→PC10850

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/57, 15/63, 9/64, A61K 38/48, C07K 16/40, C12Q 1/37

A2

(11) International Publication Number: WO 00/53774

(43) International Publication Date: 14 September 2000 (14.09.00)

(21) International Application Number: PCT/US00/06237

(22) International Filing Date: 8 March 2000 (08.03.00)

(30) Priority Data:
09/264,585 8 March 1999 (08.03.99) US

(71) Applicant *(for all designated States except US)*: NEUROCRINE BIOSCIENCES, INC. [US/US]; 10555 Science Center Drive, San Diego, CA 92121 (US).

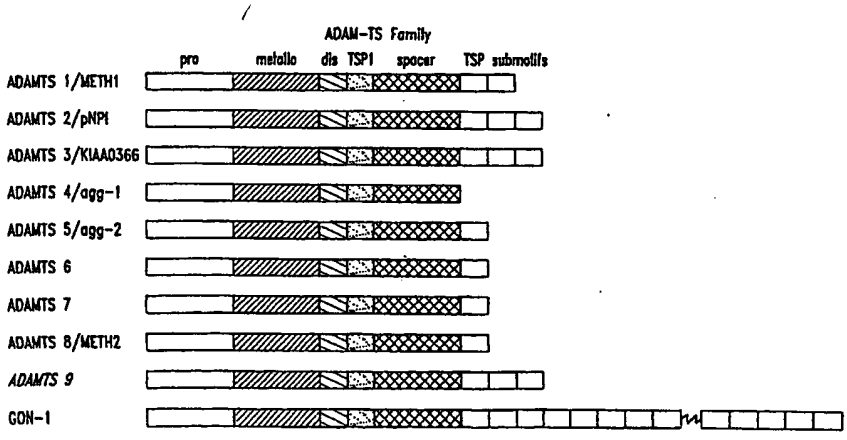
(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: KELNER, Gregory, S. [US/US]; 725 Muirlands Vista Way, La Jolla, CA 92037 (US). CLARK, Melody [US/US]; 7075 Charmant Drive #20, San Diego, CA 92122 (US). MAKI, Richard, A. [US/US]; 4175–174 Porte de Palmas, San Diego, CA 92122 (US).

(74) Agents: CHRISTIANSEN, William, T. et al.; Seed Intellectual Property Law Group PLLC, Suite 6300, 701 Fifth Avenue, Seattle, WA 98104–7092 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: METALLOPROTEINASES AND METHODS OF USE THEREFOR



(57) Abstract

Novel members of the ADAMTS family of metalloproteinases are provided, along with variants thereof and agents that modulate an activity of such metalloproteinases. The polypeptides and modulating agents may be used, for example, in the prevention and treatment of a variety of conditions associated with undesirable levels of metalloproteinase activity.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

METALLOPROTEINASES AND METHODS OF USE THEREFOR

TECHNICAL FIELD

The present invention relates generally to compositions and methods for the treatment of conditions associated with undesirable levels of metalloproteinase activity. The invention is more particularly related to metalloproteinases and agents that modulate the activity of such metalloproteinases which may be used, for example, for the therapy of diseases characterized by neuroinflammation and/or neurodegeneration, as well as autoimmune diseases, cancer and inflammation.

BACKGROUND OF THE INVENTION

The ADAMs (A Disintegrin and Metalloproteinase Domain) are a family of proteins that have both a metalloproteinase domain and disintegrin domain. The ADAMs are membrane anchored proteins that contain homology to snake venom metalloproteases (SVMPs) and disintegrins. This family of proteins now contains over 20 members that have a wide variety of important proteolytic and cell fusion functions. ADAM 17/TACE and ADAM 10/Kuz function as proteases that cleave membrane bound tumor necrosis factor (TNF) and the extracellular domain of Notch, respectively. Other ADAM family members, such as ADAM 1/fertilin α, are proteolytically processed to remove the metalloprotease domain but retain the disintegrin domain. This protein has been shown to be essential for sperm-egg cell fusion.

A closely related family called ADAMTS contains a thrombospondin domain in addition to the disintegrin and metalloproteinase domains. ADAMTS-1, for example, is expressed in association with inflammatory processes and in a cachexigenic colon carcinoma cell line (*see* Kuno et al., *J. Biol. Chem. 272*:556-562, 1997; Kuno et al., *Genomics 46*:466-471, 1997). This protein appears to be secreted from the cell and subsequently associated with the extracellular matrix (ECM).

While the function of ADAMTS-1 and many of the ADAM proteins is not known, it has been shown that ADAM 17 (TACE) processes TNF from the surface of the cell (*see* Black et al., *Nature 385*:729-733, 1997). ADAM 10 (Kuzbanian) has

also been shown to cleave TNF from the cell surface (Rosendahl et al., *J. Biol. Chem. 272*:24588-24593, 1997). ADAM 10 may be involved in the cleavage of other cell surface proteins as well. In Drosophila, ADAM 10 has been reported to cleave the cell surface proteins Notch (Pan and Rubin, *Cell 90*:271-280, 1997) and Delta (Qi et al., *Science 283*:91-94, 1999). Based largely on these results it is thought that ADAMs proteases are involved in the cleavage of proteins, including growth factors, cytokines and proteoglycans, from the cell surface.

Metalloproteinase activity has been linked to cancer metastasis. The activity of metalloproteinases can contribute to the development of neurodegeneration and inflammation as well. In order to develop agents capable of selectively modulating the activity of a metalloproteinase that contributes to a human disease, it is important to identify and characterize additional metalloproteinases, such as members of the ADAMTS family, and agents that modulate an activity of such metalloproteinases. The present invention fulfills this need and further provides other related advantages.

SUMMARY OF THE INVENTION

Briefly stated, the present invention provides ADAMTS polypeptides, and methods employing such polypeptides. Within certain aspects, isolated polynucleotides that encode an ADAMTS polypeptide are provided. Certain ADAMTS polynucleotides encode an ADAMTS polypeptide that comprises: (a) at least 50 consecutive amino acid residues of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:2, 4, 10, 14, 16, 18, 22, 24, 26 or 27; or (b) a variant of any of the foregoing amino acid sequences that differs in one or more substitutions, deletions, additions and/or insertions, wherein substitutions, if any, are present at no more than 10% of the consecutive residues of the ADAMTS protein. Such polynucleotides may, within certain embodiments, comprise a sequence recited in any one of SEQ ID NOs:1, 3, 9, 13, 15, 17, 21, 23 or 25.

Within related aspects, the present invention provides recombinant expression vectors comprising an ADAMTS polynucleotide, as well as host cells transformed or transfected with such an expression vector.

The present invention further provides isolated antisense polynucleotides complementary to at least 20 consecutive nucleotides present within an ADAMTS polynucleotide.

Within further aspects, methods are provided for preparing an ADAMTS polypeptide, comprising the steps of: (a) culturing a host cell transformed or transfected with an expression vector comprising a polynucleotide that encodes an ADAMTS polypeptide comprising: (i) at least 50 consecutive amino acid residues of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 27; or (ii) a variant of any of the foregoing amino acid sequences that differs in one or more substitutions, deletions, additions and/or insertions, wherein substitutions, if any, are present at no more than 10% of the consecutive residues of the ADAMTS protein; wherein the step of culturing is performed under conditions promoting expression of the polynucleotide sequence; and (b) recovering an ADAMTS polypeptide.

The present invention further provides isolated ADAMTS polypeptides comprising: (a) at least 50 consecutive amino acid residues of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 27; or (b) a variant of any of the foregoing amino acid sequences that differs in one or more substitutions, deletions, additions and/or insertions, wherein substitutions, if any, are present at no more than 10% of the consecutive residues of the ADAMTS protein. Such an ADAMTS polypeptide may have an ADAMTS activity that is not substantially diminished relative to the ADAMTS protein. ADAMTS polypeptide may comprise an amino acid sequence recited in any one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 27.

Within further aspects, the present invention provides pharmaceutical compositions comprising: (a) an ADAMTS polypeptide comprising: (i) at least 50 consecutive amino acid residues of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 27; or (ii) a variant of any of the foregoing amino acid sequences that differs in one or more substitutions, deletions, additions and/or insertions, wherein substitutions, if any, are

present at no more than 10% of the consecutive residues of the ADAMTS protein; and (b) a physiologically acceptable carrier.

Vaccines are also provided, comprising: (a) an ADAMTS polypeptide comprising: (i) at least 50 consecutive amino acid residues of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 27; or (ii) a variant of any of the foregoing amino acid sequences that differs in one or more substitutions, deletions, additions and/or insertions, wherein substitutions, if any, are present at no more than 10% of the consecutive residues of the ADAMTS protein; and (b) a non-specific immune response enhancer.

Within further aspects, the present invention provides isolated antibodies, or antigen-binding fragments thereof, that specifically bind to an ADAMTS polypeptide comprising a sequence recited in any one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 27.

The present invention further provides methods for screening for agents that modulate ADAMTS protein expression or activity. Within certain such aspects, methods are provided for screening for an agent that modulates ADAMTS protein expression in a cell, comprising: (a) contacting a candidate modulator with a cell expressing an ADAMTS polypeptide, wherein the polypeptide comprises: (i) at least 50 consecutive amino acid residues of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 27; or (ii) a variant of any of the foregoing amino acid sequences that differs in one or more substitutions, deletions, additions and/or insertions, wherein substitutions, if any, are present at no more than 10% of the consecutive residues of the ADAMTS protein; and (b) subsequently evaluating the effect of the candidate modulator on expression of an ADAMTS mRNA or polypeptide, and therefrom identifying an agent that modulates ADAMTS protein expression in the cell. Similar screens may be performed using a cell comprising an ADAMTS gene promoter operably linked to a reporter gene, and evaluating the effect of a candidate modulator on expression of the reporter gene.

Within further such aspects, methods are provided for screening for an agent that modulates an ADAMTS protein activity, comprising: (a) contacting a

candidate modulator with an ADAMTS polypeptide, comprising: (i) at least 50 consecutive amino acid residues of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:2, 4, 6. 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 27; or (ii) a variant of any of the foregoing amino acid sequences that differs in one or more substitutions, deletions, additions and/or insertions, wherein substitutions, if any, are present at no more than 10% of the consecutive residues of the ADAMTS protein; wherein the polypeptide has an ADAMTS activity that is not substantially diminished relative to the ADAMTS protein; and wherein the step of contacting is carried out under conditions and for a time sufficient to allow the candidate modulator to interact with the polypeptide; and (b) subsequently evaluating the effect of the candidate modulator on an ADAMTS activity of the polypeptide, and therefrom identifying an agent that modulates an activity of an ADAMTS protein.

ADAMTS polynucleotides, polypeptides and modulating agents may be used for a variety of therapeutic applications. Within certain aspects, methods are provided herein for inhibiting neuroinflammation and/or neurodegeneration in a patient, comprising administering to a patient an agent that decreases an activity of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 27. Certain such agents may inhibit expression of an endogenous ADAMTS gene or may bind to an ADAMTS protein.

Within related aspects, methods are provided for treating a patient afflicted with a condition associated with neuroinflammation and/or neurodegeneration, comprising administering to a patient a pharmaceutical composition as described above, and thereby alleviating one or more symptoms of a condition associated with neuroinflammation and/or neurodegeneration. Such conditions include Alzheimer's disease, Parkinson's disease and stroke.

Methods are further provided for treating a patient afflicted with a condition associated with cell proliferation, cell migration, inflammation and/or angiogenesis, comprising administering to a patient a pharmaceutical composition as described above and thereby alleviating one or more symptoms of a condition associated with neuroinflammation and/or neurodegeneration.

Within further aspects, methods are provided for treating a patient afflicted with an invasive tumor, a brain tumor or a brain injury, comprising administering to a patient an agent that decreases expression or activity of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 27.

Methods are further provided for modulating ADAMTS expression and/or activity in a cell, comprising contacting a cell expressing an ADAMTS polypeptide with an effective amount of an agent that modulates ADAMTS activity, wherein the ADAMTS polypeptide comprises: (i) at least 50 consecutive amino acid residues of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 27; or (ii) a variant of any of the foregoing amino acid sequences that differs in one or more substitutions, deletions, additions and/or insertions, wherein substitutions, if any, are present at no more than 10% of the consecutive residues of the ADAMTS protein; and thereby modulating ADAMTS expression and/or activity in the cell.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 presents the sequence of a polynucleotide encoding the representative human metalloproteinase ADAMTS-2 (SEQ ID NO:1).

Figure 2 presents the predicted amino acid sequence of the representative human metalloproteinase ADAMTS-2 (SEQ ID NO:2).

Figures 3A-3B present a partial sequence of a polynucleotide encoding the representative rat metalloproteinase ADAMTS-4 (SEQ ID NO:3).

Figure 4 presents a partial predicted amino acid sequence of the representative rat metalloproteinase ADAMTS-4 (SEQ ID NO:4).

Figures 5A and 5B present the sequence of a polynucleotide encoding the representative human metalloproteinase KIAA0605 (SEQ ID NO:5).

Figure 6 presents the predicted amino acid sequence of the representative human metalloproteinase KIAA0605 (SEQ ID NO:6).

Figures 7A and 7B present the sequence of a polynucleotide encoding the representative human metalloproteinase KIAA0366 (SEQ ID NO:7).

Figure 8 presents the predicted amino acid sequence of the representative human metalloproteinase KIAA0366 (SEQ ID NO:8).

Figures 9A and 9B present the sequence of a polynucleotide encoding the representative human metalloproteinase ADAMTS-3 (SEQ ID NO:9).

Figure 10 presents the predicted amino acid sequence of the representative human metalloproteinase ADAMTS-3 (SEQ ID NO:10).

Figures 11A and 11B present the sequence of a polynucleotide encoding the representative human metalloproteinase KIAA0688 (SEQ ID NO:11).

Figure 12 presents the predicted amino acid sequence of the representative human metalloproteinase KIAA0688 (SEQ ID NO:12).

Figure 13 presents the sequence of a polynucleotide encoding the representative rat metalloproteinase ADAMTS-5 (SEQ ID NO:13).

Figure 14 presents the predicted amino acid sequence of the representative rat metalloproteinase ADAMTS-5 (SEQ ID NO:14).

Figure 15 presents the sequence of a polynucleotide encoding the representative human metalloproteinase ADAMTS-4 (SEQ ID NO:15).

Figure 16 presents the predicted amino acid sequence of the representative human metalloproteinase ADAMTS-4 (SEQ ID NO:16).

Figures 17A-17G present a sequence alignment of human ADAMTS-1 (SEQ ID NO:28), ADAMTS-2 (SEQ ID NO:2), ADAMTS-3 (SEQ ID NO:10), ADAMTS-4 (SEQ ID NO:4), KIAA0688 (SEQ ID NO:12), KIAA0366 (SEQ ID NO:8) and KIAA0605 (SEQ ID NO:6).

Figure 18 presents the sequence of a polynucleotide encoding the representative bovine metalloproteinase ADAMTS-4 (SEQ ID NO:17).

Figure 19 presents the predicted amino acid sequence of the representative bovine metalloproteinase ADAMTS-4 (SEQ ID NO:18).

Figure 20 presents the sequence of a polynucleotide encoding the representative bovine metalloproteinase KIAA0688 (SEQ ID NO:19).

Figure 21 presents the predicted amino acid sequence of the representative bovine metalloproteinase KIAA0688 (SEQ ID NO:20).

Figure 22 presents the sequence of a polynucleotide encoding the representative human metalloproteinase ADAMTS-5 (SEQ ID NO:21).

Figure 23 presents the predicted amino acid sequence of the representative human metalloproteinase ADAMTS-5 (SEQ ID NO:22).

Figure 24 presents the sequence of a polynucleotide encoding the representative rat metalloproteinase ADAMTS-2 (SEQ ID NO:23).

Figure 25 presents the predicted amino acid sequence of the representative rat metalloproteinase ADAMTS-2 (SEQ ID NO:24).

Figure 26 presents the sequence of a polynucleotide encoding the representative rat metalloproteinase ADAMTS-3 (SEQ ID NO:25).

Figure 27 presents the predicted amino acid sequence of the representative rat metalloproteinase ADAMTS-3 (SEQ ID NO:26).

Figure 28 is a photograph depicting a coumassie blue-stained gel following electrophoresis of 500 micrograms brevican, previously incubated with and without ADAMTS-4 (TS-4) as indicated.

Figure 29 depicts the amino acid sequence of ADAMTS-9 (SEQ ID NO:27). The predicted signal sequence is underlined. The Zn binding, met turn, TSP 1 motif and TSP-1 like submotifs are shaded. Two potential furin cleavage sites are in parenthesis with the most likely cleavage site shaded. A potential "cysteine switch" amino acid is indicated with a star. The start of each domain is indicated with an arrow.

Figures 30A-30C illustrate the comparison of ADAMTS-9 to other ADAMTS family members. In Figure 30A, the domain structure of human ADAMTS 9 is compared to human ADAMTS 1-8, and also with the *C. elegans* GON-1 protein. The pro-domain, metalloprotease domain, disintegrin-like domain, initial TSP type 1

repeat, spacer region, and TSP1 like submotifs are outlined. Figure 30B shows the consensus sequence for Zn binding in the metalloprotease domain (SEQ ID NO:30), along with the Zn binding site for various ADAM and ADAM-TS proteins (SEQ ID Nos: 42-48, 50) that have active metalloprotease domains for comparison to ADAMTS-9 (SEQ ID NO:49). Conserved residues are shaded. Figure 30C is a dendrogram showing the phyllogenetic relationship between the protein sequence of the known ADAM-TS human family members and GON-1 from *C. elegans*.

Figure 31 is a photograph illustrating the tissue distribution pattern of ADAMTS-9 in human fetal and adult cDNA. PCR analysis of several human fetal and adult cDNAs was performed using specific primers to ADAMTS 9. Lanes 2 -16 are human adult tissue cDNAs and lanes 17 - 24 are human fetal cDNAs. Lane 25 is a no cDNA control. The expected product size for these ADAMTS 9 primers is 510 bp. The lower panel contains the same cDNA samples used as a template for PCR with G3PDH primers (expected product size is 1 kb).

Figures 32A and 32B illustrate the chrommosomal localization of human ADAMTS-9 to 3p14.3-21.1. Figure 32A is a photograph showing the results of FISH analysis in which a genomic ADAMTS 9 probe hybridized to chromosome 3p. Figure 32B shows two identogams illustrating the chromosomal position of ADAMTS-9 at 3p14.2-14.3. The International System for Human Cytogenetic Nomenclature 1995 was used.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to polypeptides comprising a member of the ADAMTS family of metalloproteinases, or a variant thereof. Such ADAMTS polypeptides are generally characterized by homology to a known ADAMTS protein, and by the presence of one or more of: (a) a disintegrin domain, (b) a zinc-dependent metalloproteinase domain, (c) an ECM domain and/or (d) a thrombospondin type I motif, which may be identified as described herein. The present invention further provides ADAMTS polynucleotides encoding such polypeptides and agents that modulate an activity of such polypeptides. ADAMTS

polypeptides, polynucleotides and/or modulating agents may generally be used for treating conditions associated with undesirable levels of metalloproteinase activity.

ADAMTS POLYNUCLEOTIDES

Any polynucleotide that encodes an ADAMTS polypeptide as described herein is encompassed by the present invention. Such polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

ADAMTS polynucleotides may comprise a native ADAMTS sequence (*i.e.*, an ADAMTS gene that can be found in an organism that is not genetically modified), or may comprise a variant of such a sequence. Native ADAMTS sequences encompassed by the present invention include DNA and RNA molecules that comprise a sequence recited in any one of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 or 25 as well as homologues thereof from other species and other native ADAMTS sequences that may be identified based on homology to a sequence recited herein. Polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions such that an ADAMTS activity of the encoded polypeptide is not diminished, relative to a native ADAMTS protein. The effect on an activity of the encoded polypeptide may generally be assessed as described herein. Preferred variants contain nucleotide substitutions, deletions, insertions and/or additions at no more than 30%, preferably at no more than 20% and more preferably at no more than 10%, of the nucleotide positions. Certain variants are substantially homologous to a native gene, or a portion or complement thereof. Such polynucleotide variants are capable of hybridizing under moderately stringent conditions to a naturally occurring DNA sequence encoding an ADAMTS polypeptide (or a complementary sequence). Suitable moderately stringent conditions include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5 X SSC, overnight; followed

by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS). Such hybridizing DNA sequences are also within the scope of this invention.

It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention.

A portion of a sequence complementary to a coding sequence (*i.e.*, an antisense polynucleotide) may also be used as a probe or to modulate gene expression. Alternatively, an antisense molecule may be designed to hybridize with a control region of a gene (*e.g.*, promoter, enhancer or transcription initiation site), and block transcription of the gene; or to block translation by inhibiting binding of a transcript to ribosomes. Antisense oligonucleotides may be synthesized directly, or cDNA constructs that can be transcribed into antisense RNA may be introduced into cells or tissues to facilitate the production of antisense RNA. Antisense oligonucleotides are preferably at least 20 nucleotides in length, preferably at least 30 nucleotides in length. A portion of a coding sequence or a complementary sequence may also be designed as a probe or primer to detect gene expression. Probes may be labeled by a variety of reporter groups, such as radionuclides and enzymes, and are preferably at least 10 nucleotides in length, more preferably at least 20 nucleotides in length and still more preferably at least 30 nucleotides in length. Primers are preferably 22-30 nucleotides in length.

ADAMTS polynucleotides may be prepared using any of a variety of techniques. For example, an ADAMTS polynucleotide may be amplified from cDNA prepared from cells that express an ADAMTS protein (*e.g.*, microglia, macrophages, myeloid cells, lymphocytes, astrocytes oligodendrocytes, glial cells, neurons, epithelial cells and/or endothelial cells). Such polynucleotides may be amplified via polymerase chain reaction (PCR). For this approach, sequence-specific primers may be designed

based on the sequences provided herein, and may be purchased or synthesized. An amplified portion may then be used to isolate a full length gene from a human genomic DNA library or from a suitable cDNA library, using well known techniques. Alternatively, a full length gene can be constructed from multiple PCR fragments. ADAMTS polynucleotides may also be prepared by synthesizing oligonucleotide components (which may be derived from sequences provided herein), and ligating components together to generate the complete polynucleotide. One other approach is to screen a library with a synthesized oligonucleotide that hybridizes to an ADAMTS gene. Libraries may generally be prepared and screened using methods well known to those of ordinary skill in the art, such as those described in Sambrook et al., *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989. It has been found, within the context of the present invention, that ADAMTS genes are expressed in glia. Accordingly, one suitable library is a microglia (*e.g.*, rat) cDNA library. Other libraries that may be employed will be apparent to those of ordinary skill in the art.

As noted above, polynucleotides comprising portions and other variants of native ADAMTS sequences are within the scope of the present invention. Such polynucleotides may generally be prepared by any method known in the art, including chemical synthesis by, for example, solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* or *in vivo* transcription of DNA sequences encoding an ADAMTS polypeptide, provided that the DNA is incorporated into a vector with a suitable RNA polymerase promoter (such as T7 or SP6). Variants may also be generated by mutagenesis or enzymatic digestion of native sequences. Certain polynucleotides may be used to prepare an encoded polypeptide, as described herein. In addition, or alternatively, a polynucleotide may be administered to a patient such that the encoded polypeptide is generated *in vivo*.

Any polynucleotide may be further modified to increase stability *in vivo*. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional

bases such as inosine, queosine and wybutosine, as well as acetyl- methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

Nucleotide sequences as described herein may be joined to a variety of other nucleotide sequences using established recombinant DNA techniques. For example, a polynucleotide may be cloned into any of a variety of cloning vectors, including plasmids, phagemids, lambda phage derivatives and cosmids. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors and sequencing vectors. In general, a vector will contain an origin of replication functional in at least one organism, convenient restriction endonuclease sites and one or more selectable markers. Other elements will depend upon the desired use, and will be apparent to those of ordinary skill in the art.

Within certain embodiments, polynucleotides may be formulated so as to permit entry into a cell of a mammal, and expression therein. Those of ordinary skill in the art will appreciate that there are many ways to achieve expression of a polynucleotide in a target cell, and any suitable method may be employed. For example, a polynucleotide may be incorporated into a viral vector such as, but not limited to, adenovirus, adeno-associated virus, retrovirus, or vaccinia or other pox virus (*e.g.*, avian pox virus). Techniques for incorporating DNA into such vectors are well known to those of ordinary skill in the art. A retroviral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of transduced cells) and/or a targeting moiety, such as a gene that encodes a ligand for a receptor on a specific target cell, to render the vector target specific. Targeting may also be accomplished using an antibody, by methods known to those of ordinary skill in the art.

Other formulations for polynucleotides for therapeutic purposes include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*i.e.*, an artificial membrane vesicle). The preparation and use of such systems is well known in the art.

ADAMTS POLYPEPTIDES

As used herein, the term "ADAMTS polypeptide" encompasses amino acid chains of any length. For example, an ADAMTS polypeptide may comprise a full length endogenous (*i.e.*, native) ADAMTS protein. Such an ADAMTS polypeptide may consist entirely of a native ADAMTS sequence, or may contain additional heterologous sequences. Native ADAMTS proteins may generally be identified based on sequence homology to known ADAMTS protein sequences, such as the representative sequences provided herein, particularly within disintegrin, metalloproteinase and/or thrombospondin motifs. In general, a protein is considered to be an ADAMTS protein if at least 20 consecutive amino acid residues, preferably 40 consecutive amino acids, are identical to a known ADAMTS protein. Alternatively, or in addition, an ADAMTS protein may comprise at least 100 consecutive amino acids that are substantially similar to residues within a known ADAMTS metalloproteinase. "Substantial similarity," as used herein, refers to a sequence that is at least 50% identical, and preferably at least 80% identical.

An ADAMTS protein further comprises one or more of: (a) a disintegrin domain, (b) a zinc-dependent metalloproteinase domain and/or (c) a thrombospondin type I motif; and displays at least one, activity characteristic of such a domain or motif. In general a disintegrin domain serves as an integrin binding loop and has a sequence similar to AVN(E/D)CD (SEQ ID NO:29). Disintegrin domains can also contain the sequence RGD. The metalloproteinase domain is based on the presence of an extended catalytic site consensus sequence (HEXXHXXGXXHD; SEQ ID NO:30). It is thought that the three histidines bind the zinc, the glutamic acid is the catalytic base and the glycine allows an important structural turn (Stocker et al., *Protein Science 4*:823-840, 1995). The thrombospondin domain contains the sequence motif CSRTCG (SEQ ID NO:31).

Another domain that may be present within an ADAMTS protein is a domain that binds to the extracellular matrix. This has been referred to as the ECM domain and has the semiconserved sequence FREEQC (SEQ ID NO:32).

In certain embodiments, amino acid residues within a "substantially similar" region may contain primarily or entirely conservative substitutions. A conservative substitution is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity on polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his.

An ADAMTS polypeptide may comprise a portion of a native ADAMTS protein. Such a portion is preferably at least 20 consecutive amino acid residues in length, more preferably at least 50 consecutive amino acid residues in length. Within certain embodiments, the portion retains an ADAMTS activity that is not substantially diminished relative to the full length ADAMTS protein. Certain ADAMTS polypeptides comprise a sequence recited in any one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 27.

Alternatively, an ADAMTS polypeptide may comprise a variant of an ADAMTS protein or portion thereof. A "variant" is a polypeptide that differs in sequence from a native ADAMTS protein only in substitutions, deletions, insertions and/or additions. Within certain embodiments, substitutions are made (if at all) at no more than 30%, preferably at no more than 20% and more preferably at no more than 10% of residues within a portion of a native ADAMTS protein, as described above. Substitutions are preferably conservative, as described above. Substitutions, deletions and/or amino acid additions may be made at any location(s) in the polypeptide,

provided that the modification does not diminish at least one ADAMTS activity. Thus, a variant may comprise only a portion of a native ADAMTS sequence. In addition, or alternatively, variants may contain additional amino acid sequences (such as, for example, linkers, tags and/or ligands), preferably at the amino and/or carboxy termini. Such sequences may be used, for example, to facilitate purification, detection or cellular uptake of the polypeptide.

Certain variants retain an activity of the native ADAMTS protein. In other words, the variant has a metalloproteinase activity; (2) functions as an integrin ligand (*i.e.*, binds to an integrin), as determined by any standard binding assay; and/or (3) retains a functional thrombospondin motif. Such a variant may have an ADAMTS activity that is not substantially diminished relative to the ADAMTS protein. In other words, the ADAMTS activity of the variant may be enhanced or unchanged, relative to the native protein, or may be diminished by less than 50%, and preferably less than 20%, relative to the native protein.

Also encompassed by the present invention are splice variants of an ADAMTS protein. Such variants may have one or more of the domains described herein deleted, or one or more such domains may be replaced by a domain providing a different function. Such splice variants may be identified using amplification or hybridization techniques described herein.

Dominant negative forms of ADAMTS proteins are also provided. Such forms include fragments and variants of an ADAMTS protein that, when introduced to a cell expressing a native ADAMTS protein, inhibit an activity of the native protein. Inhibition of ADAMTS protein activity may be assessed as described herein.

In general, ADAMTS polypeptides may be prepared using any of a variety of techniques that are well known in the art. For example, polypeptides of the present invention may be prepared by expression of recombinant DNA encoding the polypeptide in cultured host cells. Preferably, the host cells are bacteria, yeast, insect or mammalian cells. The recombinant DNA may be cloned into any expression vector suitable for use within the host cell and transfected into the host cell using techniques well known to those of ordinary skill in the art. An expression vector generally contains

a promoter sequence that is active in the host cell. A tissue specific promoter may also be used, as long as it is activated in the target cell. Preferred promoters express the polypeptide at high levels.

Optionally, the construct may contain an enhancer, a transcription terminator, a poly(A) signal sequence, a bacterial or mammalian origin of replication and/or a selectable marker, all of which are well known in the art. Enhancer sequences may be included as part of the promoter region used or separately. Transcription terminators are sequences that stop RNA polymerase-mediated transcription. The poly(A) signal may be contained within the termination sequence or incorporated separately. A selectable marker includes any gene that confers a phenotype on the host cell that allows transformed cells to be identified. Such markers may confer a growth advantage under specified conditions. Suitable selectable markers for bacteria are well known and include resistance genes for ampicillin, kanamycin and tetracycline. Suitable selectable markers for mammalian cells include hygromycin, neomycin, genes that complement a deficiency in the host (*e.g.* thymidine kinase and TK^- cells) and others well known in the art.

ADAMTS polypeptides may be expressed in transfected cells by culturing the cell under conditions promoting expression of the transfected polynucleotide. Appropriate conditions will depend on the specific host cell and expression vector employed, and will be readily apparent to those of ordinary skill in the art. For commercially available expression vectors, the polypeptide may generally be expressed according to the manufacturer's instructions. Expressed polypeptides of this invention are generally isolated in substantially pure form. Preferably, the polypeptides are isolated to a purity of at least 80% by weight, more preferably to a purity of at least 95% by weight, and most preferably to a purity of at least 99% by weight. In general, such purification may be achieved using, for example, the standard techniques of ammonium sulfate fractionation, SDS-PAGE electrophoresis, and/or affinity chromatography.

Such techniques may be used to prepare native polypeptides or variants thereof. For example, variants of a native polypeptide may generally be prepared from

polynucleotide sequences modified via standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis, and sections of the DNA sequence may be removed to permit preparation of truncated polypeptides. Portions and other variants having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may also be generated by synthetic means, using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. *See* Merrifield, *J. Am. Chem. Soc. 85*:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Applied BioSystems, Inc. (Foster City, CA), and may be operated according to the manufacturer's instructions.

In general, polypeptides and polynucleotides as described herein are isolated. An "isolated" polypeptide or polynucleotide is one that is removed from its original environment. For example, a naturally-occurring protein is isolated if it is separated from some or all of the coexisting materials in the natural system. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of the natural environment.

Evaluation of ADAMTS Activity

As noted above, native ADAMTS proteins and certain variants thereof possess ADAMTS activity. In other words, such polypeptides (1) possess metalloproteinase activity; (2) are capable of interacting with integrin and/or (3) retain a functional thrombospondin motif. Metalloproteinase activity may generally be evaluated by combining an ADAMTS polypeptide with a suitable substrate, and detecting proteinase activity using any standard technique (*e.g.,* Western blot analysis). In general, a variant of an ADAMTS protein that contains a metalloproteinase domain is said to retain metalloproteinase activity if it displays metalloproteinase activity that is not substantially diminished relative to the metalloproteinase activity of the native

ADAMTS protein. In other words, such activity may be enhanced, unchanged or diminished by less than 10%, relative to the activity of the native ADAMTS protein.

The ability of an ADAMTS protein variant to interact with integrin may be assessed using standard binding assays to detect interaction with a purified recombinant integrin or a cell expressing one or more integrins, either naturally or as a result of transfection with genes encoding an integrin (*see* Almeida et al., *Cell 81*:1095-1104, 1995; Chen et al., *J. Cell Biol. 144*:549-561, 1999). Antibodies against various integrins can also be used to interfere with disintegrin-integrin binding and used to further demonstrate specificity of the interaction. In general, a variant of an ADAMTS protein is said to retain the ability to interact with an integrin if such interaction is not substantially diminished relative to the interaction between a native ADAMTS protein and the integrin. In other words, the level of such an interaction may be enhanced, unchanged or diminished by less than 10%, relative to the activity of the native ADAMTS protein.

Thrombospondins have been shown to function in cell adhesion, cell migration, cell proliferation and angiogenesis. A functional thrombospondin motif may be confirmed based on any assay designed to assess such a function. For examples, an ADAMTS protein may inhibit endothelial cell migration, or may inhibit angiogenesis (*e.g.*, in a rat cornea model; *see* Nishimori et al., *Oncogene 15*:2145-2150, 1997). Alternatively, a functional thrombospondin motif may be detected using an assay to measure binding to CD36 (*see* Dawson et al., *J. Cell. Biol. 138*:707-717, 1997). Within any such assay, a variant of an ADAMTS protein is said to have a functional thrombospondin motif if the detected thrombospondin function is not substantially diminished relative to that of the native ADAMTS protein. In other words, the function may be enhanced, unchanged or diminished by less than 10%, relative to that of the native ADAMTS protein.

ADAMTS Polypeptide Modulating Agents

The present invention further provides agents capable of modulating ADAMTS activity. Such agents may function by modulating ADAMTS transcription

or translation, by stabilizing or destabilizing an ADAMTS protein, or by directly inhibiting or enhancing an activity of an ADAMTS protein. Alternatively, an agent may interact with a substrate for the metalloproteinase or with an integrin involved in and interaction with the disintegrin domain of an ADAMTS protein. Preferably, a modulating agent has a minimum of side effects and is non-toxic. For some applications, agents that can penetrate cells or that are targeted to interstitial spaces are preferred.

Modulating agents include substances that selectively bind to an ADAMTS protein. Such substances include antibodies and antigen-binding fragments thereof (*e.g.*, $F(ab)_2$, Fab, Fv, V_H or V_K fragments), as well as single chain antibodies, multimeric monospecific antibodies or fragments thereof and bi- or multi-specific antibodies and fragments thereof. Antibodies that bind to an ADAMTS protein may be polyclonal or monoclonal, and are specific for an ADAMTS polypeptide (*i.e.*, bind to such a peptide detectable within any appropriate binding assay, and do not bind to an unrelated protein in a similar assay under the same conditions). Preferred antibodies are those antibodies that function as modulating agents to inhibit or block an ADAMTS activity *in vivo*. Antibodies may also be employed within assays for detecting the level of ADAMTS protein within a sample.

Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art (*see, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988). In one such technique, an immunogen comprising the polypeptide is initially injected into a suitable animal (*e.g.*, mice, rats, rabbits, sheep and goats), preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of

producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction.

Once a cell line, such as a hybridoma, expressing an antibody that specifically binds to an ADAMTS protein has been obtained, other chimeric antibodies and fragments thereof as described herein may be prepared. Using well known techniques, a cDNA molecule encoding the antibody may be identified.

Other modulating agents include peptides, and nonpeptide mimetics thereof, that specifically interact with one or more regions of an ADAMTS polypeptide. Such agents may generally be identified using any well known binding assay, such as a representative assay provided herein. For example, such modulating agents may be isolated using well known techniques to screen substances from a variety of sources, such as plants, fungi or libraries of chemicals, small molecules or random peptides.

Other modulating agents may function by inhibiting or enhancing transcription or translation of an ADAMTS gene. For example, modulating agents may include antisense polynucleotides (DNA or RNA), which inhibit the transcription of a native ADAMTS protein. cDNA constructs that can be transcribed into antisense RNA may also be introduced into cells of tissues to facilitate the production of antisense RNA. Antisense technology can generally be used to control gene expression through triple-helix formation, which compromises the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors or regulatory molecules (*see* Gee et al., *In* Huber and Carr, *Molecular and Immunologic Approaches*, Futura Publishing Co. (Mt. Kisco, NY; 1994). Alternatively, an antisense molecule may be designed to hybridize with a control region of a gene (*e.g.*, promoter, enhancer or transcription initiation site), and block transcription of the gene; or to block translation by inhibiting binding of a transcript to ribosomes. Antisense polynucleotides are generally at least 10 nucleotides in length, more preferably at least 20 nucleotides in length and still more preferably at least 30 nucleotides in length.

Other agents may modulate transcription by interacting with an ADAMTS promoter. Such agents may be identified using standard assays, following isolation of an endogenous ADAMTS gene promoter region. One method for identifying a promoter region uses a PCR-based method to clone unknown genomic DNA sequences adjacent to a known cDNA sequence. This approach may generate a 5' flanking region, which may be subcloned and sequenced using standard methods. Primer extension and/or RNase protection analyses may be used to verify the transcriptional start site deduced from the cDNA.

To define the boundary of the promoter region, putative promoter inserts of varying sizes may be subcloned into a heterologous expression system containing a suitable reporter gene without a promoter or enhancer may be employed. Internal deletion constructs may be generated using unique internal restriction sites or by partial digestion of non-unique restriction sites. Constructs may then be transfected into cells that display high levels of ADAMTS protein expression In general, the construct with

the minimum 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter.

To evaluate the effect of a candidate agent on ADAMTS gene transcription, a promoter or regulatory element thereof may be operatively linked to a reporter gene. Such a construct may be transfected into a suitable host cell, which may be used to screen, for example, a combinatorial small molecule library. Briefly, cells are incubated with the library (*e.g.*, overnight). Cells are then lysed and the supernatant is analyzed for reporter gene activity according to standard protocols. Compounds that result in a decrease in reporter gene activity are inhibitors of ADAMTS gene transcription.

For modulating agents that act directly on an ADAMTS protein, an initial screen to assess the ability of candidate agents to bind to such a protein may be employed, although such binding is not essential for a modulating agent. For identifying agents that bind to an ADAMTS polypeptide, any of a variety of binding assays may be employed, such as standard affinity techniques and yeast two-hybrid screens. In general, the amount of candidate modulator added in such screens ranges from about 1 pM to 1 μM. An antibody or other modulating agent is said to "specifically bind" to an ADAMTS polypeptide if it reacts at a detectable level with such a polypeptide and does not react detectably with unrelated polypeptides. Such antibody binding properties may be assessed using, for example, an ELISA.

Screens for modulating agents that increase the rate of ADAMTS protein synthesis or stabilize ADAMTS protein may be readily performed using well known techniques that detect the level of ADAMTS protein or mRNA. Suitable assays include RNA protection assays, *in situ* hybridization, ELISAs, Northern blots and Western blots. Such assays may generally be performed using standard methods (*see* Sambrook et al., *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989). For example, to detect mRNA encoding ADAMTS protein, a nucleic acid probe complementary to all or a portion of an ADAMTS gene sequence may be employed in a Northern blot analysis of mRNA prepared from suitable cells (*e.g.*, brain, lung, heart, spleen, spinal cord, testis, astrocytes or microglia).

To detect ADAMTS protein, a reagent that binds to the protein (typically an antibody) may be employed within an ELISA or Western assay. Following binding, a reporter group suitable for direct or indirect detection of the reagent is employed (*i.e.*, the reporter group may be covalently bound to the reagent or may be bound to a second molecule, such as Protein A, Protein G, immunoglobulin or lectin, which is itself capable of binding to the reagent). Suitable reporter groups include, but are not limited to, enzymes (*e.g.*, horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. Such reporter groups may be used to directly or indirectly detect binding of the reagent to a sample component using standard methods known to those of ordinary skill in the art.

To use such assays for identifying a modulating agent, the level of ADAMTS protein or mRNA is evaluated in cells (*e.g.*, astrocytes or microglia) treated with one or more candidate modulating agents. An increase or decrease in ADAMTS levels may be measured by evaluating ADAMTS mRNA and/or protein in the presence and absence of candidate modulating agent. In general, the amount of candidate modulator added in such screens ranges from about 1 pM to 1 μM. A candidate that results in a statistically significant change in the level of ADAMTS mRNA and/or protein is a modulating agent.

Modulating agents that decrease ADAMTS levels generally inhibit ADAMTS activity. To further evaluate the effect on ADAMTS activity, an assay may be performed as described above in the presence and absence of modulating agent. Agents that bind to a substrate of an ADAMTS protein domain may also be identified using such assays. Modulating agents may generally be administered by addition to a cell culture or by the methods described below for *in vivo* administration.

ADAMTS POLYPEPTIDE AND MODULATING AGENT MODIFICATION AND FORMULATIONS

An ADAMTS polypeptide or modulating agent as described herein may, but need not, be linked to one or more additional molecules. In particular, as discussed below, it may be beneficial for certain applications to link multiple polypeptides and/or modulating agents (which may, but need not, be identical) to a support material, such as

a polymeric matrix or a bead or other particle, which may be prepared from a variety of materials including glass, plastic or ceramics. For certain applications, biodegradable support materials are preferred.

Suitable methods for linking an ADAMTS polypeptide or modulating agent to a support material will depend upon the composition of the support and the intended use, and will be readily apparent to those of ordinary skill in the art. Attachment may generally be achieved through noncovalent association, such as adsorption or affinity or, preferably, via covalent attachment (which may be a direct linkage or may be a linkage by way of a cross-linking agent).

It may be beneficial for certain applications to link an ADAMTS polypeptide or modulating agent to a targeting agent to facilitate targeting to one or more specific tissues. As used herein, a "targeting agent," may be any substance (such as a compound or cell) that, when linked to a polypeptide or modulating agent enhances the transport of the polypeptide or modulating agent to a target tissue, thereby increasing the local concentration. Targeting agents include antibodies or fragments thereof, receptors, ligands and other molecules that bind to cells of, or in the vicinity of, the target tissue. Known targeting agents include serum hormones, antibodies against cell surface antigens, lectins, adhesion molecules, tumor cell surface binding ligands, steroids, cholesterol, lymphokines, fibrinolytic enzymes and those drugs and proteins that bind to a desired target site. An antibody targeting agent may be an intact (whole) molecule, a fragment thereof, or a functional equivalent thereof. Linkage is generally covalent and may be achieved by, for example, direct condensation or other reactions, or by way of bi- or multi-functional linkers. Within other embodiments, it may also be possible to target a polynucleotide encoding a polypeptide or modulating agent to a target tissue, thereby increasing the local concentration. Such targeting may be achieved using well known techniques, including retroviral and adenoviral infection. To treat a patient afflicted with certain conditions (*e.g.*, neurodegenerative conditions), it may be beneficial to deliver an ADAMTS polypeptide, polynucleotide or modulating agent to the intracellular space. Such targeting may be achieved using well known

techniques, such as through the use of polyethylene glycol or liposomes, as described in Turrens, *Xenobiotica 21*:1033-1040, 1991.

For certain embodiments, it may be beneficial to also, or alternatively, link a drug to a polypeptide or modulating agent. As used herein, the term "drug" refers to any bioactive agent intended for administration to a mammal to prevent or treat a disease or other undesirable condition.

Within certain aspects of the present invention, one or more polypeptides, polynucleotides or modulating agents as described herein may be present within a pharmaceutical composition or vaccine. A pharmaceutical composition further comprises one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Vaccines may comprise one or more such compounds and a non-specific immune response enhancer. A non-specific immune response enhancer may be any substance that enhances an immune response to an exogenous antigen. Examples of non-specific immune response enhancers include adjuvants and liposomes.

To prepare a pharmaceutical composition, an effective amount of one or more polypeptides, polynucleotides and/or modulating agents is mixed with a suitable pharmaceutical carrier. Solutions or suspensions used for parenteral, intradermal, subcutaneous or topical application can include, for example, a sterile diluent (such as water), saline solution, fixed oil, polyethylene glycol, glycerin, propylene glycol or other synthetic solvent; antimicrobial agents (such as benzyl alcohol and methyl parabens); antioxidants (such as ascorbic acid and sodium bisulfite) and chelating agents (such as ethylenediaminetetraacetic acid (EDTA)); buffers (such as acetates, citrates and phosphates). If administered intravenously, suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, polypropylene glycol and mixtures thereof. In addition, other pharmaceutically active ingredients and/or suitable excipients such as salts, buffers and stabilizers may, but need not, be present within the composition.

A pharmaceutical composition is generally formulated and administered to exert a therapeutically useful effect while minimizing undesirable side effects. The

number and degree of acceptable side effects depend upon the condition for which the composition is administered. For example, certain toxic and undesirable side effects that are tolerated when treating life-threatening illnesses, such as tumors, would not be tolerated when treating disorders of lesser consequence. The concentration of active component in the composition will depend on absorption, inactivation and excretion rates thereof, the dosage schedule and the amount administered, as well as other factors that may be readily determined by those of skill in the art.

A polypeptide, polynucleotide or modulating agent may be prepared with carriers that protect it against rapid elimination from the body, such as time release formulations or coatings. Such carriers include controlled release formulations, such as, but not limited to, implants and microencapsulated delivery systems, and biodegradable, biocompatible polymers, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, polyorthoesters, polylactic acid and others known to those of ordinary skill in the art. Such formulations may generally be prepared using well known technology and administered by, for example. oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a polynucleotide, polypeptide or modulating agent dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane. Preferably the formulation provides a relatively constant level of modulating agent release. The amount of active component contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Pharmaceutical compositions of the present invention may be administered in a manner appropriate to the disease to be treated (or prevented). Administration may be effected by incubation of cells *ex vivo* or *in vivo*, such as by topical treatment, delivery by specific carrier or by vascular supply. Appropriate dosages and a suitable duration and frequency of administration will be determined by such factors as the condition of the patient, the type and severity of the patient's disease and the method of administration. In general, an appropriate dosage and treatment regimen provides the polypeptide, polynucleotide and/or modulating agent(s) in an

amount sufficient to provide therapeutic and/or prophylactic benefit (*i.e.*, an amount that ameliorates the symptoms or treats or delays or prevents progression of the condition). The precise dosage and duration of treatment is a function of the disease being treated and may be determined empirically using known testing protocols or by testing the compositions in model systems known in the art and extrapolating therefrom. Dosages may also vary with the severity of the condition to be alleviated. The composition may be administered one time, or may be divided into a number of smaller doses to be administered at intervals of time. In general, the use of the minimum dosage that is sufficient to provide effective therapy is preferred. Patients may generally be monitored for therapeutic effectiveness using assays suitable for the condition being treated or prevented, which will be familiar to those of ordinary skill in the art, and for any particular subject, specific dosage regimens may be adjusted over time according to the individual need.

For pharmaceutical compositions comprising polynucleotides, the polynucleotide may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid, bacterial and viral expression systems, and colloidal dispersion systems such as liposomes. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal, as described above). The DNA may also be "naked," as described, for example, in Ulmer et al., *Science 259*:1745-1749, 1993.

Various viral vectors that can be used to introduce a nucleic acid sequence into the targeted patient's cells include, but are not limited to, vaccinia or other pox virus, herpes virus, retrovirus, or adenovirus. Techniques for incorporating DNA into such vectors are well known to those of ordinary skill in the art. Preferably, the retroviral vector is a derivative of a murine or avian retrovirus including, but not limited to, Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A retroviral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of transduced cells) and/or a gene that

encodes the ligand for a receptor on a specific target cell (to render the vector target specific).

Viral vectors are typically non-pathogenic (defective), replication competent viruses, which require assistance in order to produce infectious vector particles. This assistance can be provided, for example, by using helper cell lines that contain plasmids that encode all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR, but that are missing a nucleotide sequence which enables the packaging mechanism to recognize an RNA transcript for encapsulation. Such helper cell lines include (but are not limited to) Ψ2, PA317 and PA12. A retroviral vector introduced into such cells can be packaged and vector virion produced. The vector virions produced by this method can then be used to infect a tissue cell line, such as NIH 3T3 cells, to produce large quantities of chimeric retroviral virions.

Another targeted delivery system for polynucleotides is a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*i.e.*, an artificial membrane vesicle). RNA, DNA and intact virions can be encapsulated within the aqueous interior and delivered to cells in a biologically active form. The preparation and use of liposomes is well known to those of ordinary skill in the art.

THERAPEUTIC APPLICATIONS

As noted above, ADAMTS polynucleotides, polypeptides and modulating agents may generally be used for the therapy of diseases characterized by neuroinflammation or neurodegeneration. In general, ADAMTS metalloproteinases are believed to function in cleaving proteins from cell surfaces (which may be surfaces of cells that synthesize the metalloproteinase or other cells). Pharmaceutical compositions as provided herein may be administered to a patient, alone or in combination with other therapies, to treat or prevent neurodegenerative diseases such as Alzheimer's disease,

Parkinson's disease or stroke. Pharmaceutical compositions provided herein may also be beneficial for therapy of conditions related to cell proliferation, cell migration, inflammation or angiogenesis. Such conditions include cancer, arthritis and autoimmune diseases.

Modulation of an ADAMTS function, either *in vitro* or *in vivo*, may generally be achieved by administering a modulating agent that inhibits ADAMTS transcription, translation or activity. In some instances, however, the ADAMTS activity may be lower than is desired. In such cases, polynucleotides, polypeptides and/or modulating agents that enhance ADAMTS activity may be administered. The activity of an endogenous ADAMTS protein within a cell may be increased by, for example, inducing expression of the ADAMTS gene and/or administering a modulating agent that enhances ADAMTS activity. Each of these methods may be performed using mammalian cells in culture or within a mammal, such as a human.

Certain ADAMTS polypeptides may be used to cleave the proteoglycan brevican. Brevican is a brain specific proteoglycan. The secreted form of brevican is upregulated in response to CNS injury and has been implicated in reactive gliosis, and a cleaved form may be important for tumor invasion (*see* Zhang et al., *J. Neuroscience 18*:2370-76, 1998). Thus, brevican cleavage appears to be important in brain injury and gliomas. Modulating agents that inhibit the ability of such ADAMTS polypeptides to cleave brevican may be used to treat brain injuries, brain tumors and other invasive tumors.

Routes and frequency of administration, as well as dosage, will vary from individual to individual, and may be readily established using standard techniques. In general, the pharmaceutical compositions and vaccines may be administered by injection (*e.g.*, intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration) or orally. A suitable dose is an amount of a compound that, when administered as described above, is capable of causing modulation of an ADAMTS activity that leads to an improved clinical outcome (*e.g.*, more frequent remissions, complete or partial or longer disease-free survival) in vaccinated patients as compared to non-vaccinated patients. In general, an appropriate dosage and treatment regimen

provides the active compound(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Such a response can be monitored by establishing an improved clinical outcome (*e.g.*, more frequent remissions, complete or partial, or longer disease-free survival) in treated patients as compared to non-treated patients. In general, suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

DIAGNOSTIC APPLICATIONS

In a related aspect of the present invention, kits for detecting ADAMTS proteins are provided. Such kits may be designed for detecting the level of ADAMTS protein or nucleic acid encoding an ADAMTS protein within a sample. In general, the kits of the present invention comprise one or more containers enclosing elements, such as reagents or buffers, to be used in the assay. A kit for detecting the level of ADAMTS protein or nucleic acid typically contains a reagent that binds to the ADAMTS protein, DNA or RNA. To detect nucleic acid, the reagent may be a nucleic acid probe or a PCR primer. To detect protein, the reagent is typically an antibody. A kit may also contain a reporter group suitable for direct or indirect detection of the reagent as described above.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1

Preparation of Novel ADAMTS Family Members

This Example illustrates the cloning of cDNA molecules encoding members of the ADAMTS family of metalloproteinases based on induction of expression in rat glial cells by aggregated beta amyloid.

Subtractive hybridization was performed as described (Kelner and Maki. *Methods in Molecular Medicine, vol 22: Neurodegeneration Methods and Protocols*, Eds J. Harry and H.A. Tilson, Human Press Inc., Totowa, NJ). Briefly, rat glial cells were cultured and treated with aggregated beta amyloid. After 24 hours, RNA was prepared from these cells and from control cells that were not treated with beta amyloid. Genes expressed in the activated cells but not the control cells were sequenced. This screen identified rat ADAMTS-3 (cDNA and encoded protein sequences shown in Figure 26 (SEQ ID NO:25) and Figure 27 (SEQ ID NO:26), respectively). The rat cDNA was used to screen a human cDNA library and resulted in the isolation of human ADAMTS-3. ADAMTS-3 is 2,866 nucleotides in length (Figures 9A and 9B; SEQ ID NO:9) and codes for a putative protein that is 955 amino acids in length (Figure 10; SEQ ID NO:10). ADAMTS-3 contains a metalloproteinase domain, a disintegrin domain, thrombospondin motifs and an ECM domain.

Example 2

Preparation of Novel ADAMTS Family Members using Degenerate PCR

This Example illustrates the use of degenerate PCR to clone partial cDNA molecules encoding members of the ADAMTS family of metalloproteinases.

PCR was performed using rat microglia cDNA and degenerate oligonucleotides derived from an analysis of the sequence from ADAMTS-1 and ADAMTS-3. Degenerate primers were designed based on common sequences between

these two genes. The original degenerate primers were designed based on a small region of these two genes that was cloned. One primer had the sequence 5'-TTYMGNGARGARCARTGY-3' (SEQ ID NO:33), while the other primer had the sequence 5'-RCANAYNCCRCAYTTRTC-3' (SEQ ID NO:34). The PCR conditions were annealing at 47°C for 1 minute, 72°C extension for 2 minutes and 94°C denaturation for 30 seconds.

Following PCR samples were fractionated by gel electrophoresis and fragments of the expected size were cloned into the vector pCRScript and sequenced. One amplified cDNA molecule was designated rat ADAMTS-2 (Figure 24; SEQ ID NO:23), and the encoded protein has the predicted sequence shown in Figure 25 (SEQ ID NO:24). This cDNA was used to screen a human cDNA library, from which human ADAMTS-2 was identified. Human ADAMTS-2 has the sequence shown in Figure 1 (SEQ ID NO:1), and appears to encode the protein recited in Figure 2 (SEQ ID NO:2).

Rat ADAMTS-4 was isolated using the PCR approach and is a polynucleotide having the sequence shown in Figures 3A and 3B (SEQ ID NO:3), which appears to encode the protein recited in Figure 4 (SEQ ID NO:4). For rat ADAMTS-4 the metalloproteinase domain begins at amino acid 260(R), the disintegrin domain begins at residue 487(Q), a thrombospondin motif begins at residue 570(W) and an ECM domain begins at residue 621(C). The rat ADAMTS-4 sequence was used to screen a human cDNA library and human ADAMTS-4 was isolated. Human ADAMTS-4 is 1455 nucleotides in length (Figure 15; SEQ ID NO:15) and codes for a putative protein that is 485 amino acids in length (Figure 16; SEQ ID NO:16). The disintegrin domain in human ADAMTS-4 begins at amino acid 39(E), the start of the first thrombospondin repeat is at amino acid 124(W) and the start of another thrombospondin repeat is at amino acid 479(C). Bovine ADAMTS-4 cDNA has the sequence shown in Figure 18 (SEQ ID NO:17), encoding the predicted amino acid sequence shown in Figure 19 (SEQ ID NO:18).

Rat ADAMTS-5 is a cDNA molecule with the sequence shown in Figure 13 (SEQ ID NO:13), encoding the amino acid sequence shown in Figure 14 (SEQ ID

NO:14). The human ADAMTS cDNA and protein sequences are shown in Figure 22 (SEQ ID NO:21) and Figure 23 (SEQ ID NO:22), respectively.

ADAMTS-4 was further shown to cleave the brain-specific proteoglycan brevican. Five hundred micrograms of purified brevican was cleaved with 500 micrograms of human ADAMTS-4 and incubated overnight at 37°C. The cleavage reaction was vacuum dried and resuspended in SDS sample loading dye for running on a 4-20% SDS polyacrylamide gel. Equal amounts of cleaved and uncleaved brevican were added to the gel. After electrophoresis the gel was stained with Coumassie Blue to visualize the protein bands. The results, presented in Figure 30, show that brevican is cleaved upon incubation with ADAMTS-4.

Example 3

Identification of ADAMTS Family Members using Database Searches

This Example illustrates the use of database searches to identify cDNA molecules encoding members of the ADAMTS family of metalloproteinases.

To identify additional members of the ADAMTS family, the GenBank database was searched for sequences similar to ADAMTS-1 and ADAMTS-3. This search retrieved KIAA0605 (Figures 5A and 5B; SEQ ID NO:5), which appears to encode a protein of 951 amino acids (Figure 6; SEQ ID NO:6). The coding sequence contains thrombospondin motifs, but no metalloproteinase or disintegrin domains have been identified. A thrombospondin motif begins with amino acid 50(W). Six additional thrombospondin motifs were found beginning with amino acid 568(K). The domain that binds to the extracellular matrix begins with amino acid 105(C).

Also retrieved was KIAA0366 (Figures 7A and 7B; SEQ ID NO:7), which appears to encode a protein of 951 amino acids (Figure 8; SEQ ID NO:8), including metalloproteinase and disintegrin domains, as well as thrombospondin motifs. For KIAA0366, the metalloproteinase domain begins with amino acid 241(T), the disintegrin domain begins with amino acid 460(D), a thrombospondin domain is present beginning at position 544(W) and another thrombospondin repeat occurs at position

842(W). The ECM domain begins at amino acid 597(C) and contains the semiconserved sequence FREEQC (SEQ ID NO:32). KIAA0366 does not appear to have a transmembrane domain, and therefore is likely to encode a secreted protein.

An additional sequence identified in this search was KIAA0688 (Figures 11A and 11B; SEQ ID NO:11), which appears to encode the protein shown in Figure 12 and SEQ ID NO:12. This gene codes for a protein with a metalloproteinase domain beginning at amino acid 245(R), a disintegrin domain beginning at amino acid 465(E), a thrombospondin motif at position 550(W), an ECM domain at position 601(C) and two additional thrombospondin motifs at position 905(W). A bovine KIAA0688 cDNA sequence is shown in Figure 20 (SEQ ID NO:19), and the predicted amino acid sequence of the encoded protein is shown in Figure 21 (SEQ ID NO:20).

Figures 17A-17G present an alignment of the ADAMTS protein sequences described herein, along with ADAMTS-1.

Example 4

Identification and Characterization of ADAMTS-9

This Example illustrates the cloning and characterization of the ADAM-TS/metallospondin family member designated herein as ADAMTS-9.

A small fragment of the rat ADAMTS-9 gene was initially cloned from a beta amyloid-treated (35 μg/ml aggregated Aβ 1-42) rat astrocyte cDNA library. DNA sequence analysis was performed using a PCR procedure employing fluorescent dideoxynucleotides and a model ABI-377 automated sequencer (PE Biosystem). BLAST sequence analysis revealed low homology at the protein level to the spacer region of the murine ADAMTS-1 gene.

This clone was labeled with [α-^{32}P]dCTP using the Prime It II kit (Stratagene) and used to screen a human spinal cord phage library (Clontech) according to the manufacturer's instructions. Positive plaques were purified and lambda DNA prepared (Qiagen). Several overlapping clones were sequenced that had homology to the original rat clone. In order to determine the 5′ and 3′ ends of the gene RACE (rapid

amplification of cDNA ends) analysis was performed using Marathon Ready placenta and fetal cDNA libraries (Clontech) with SMART primers (Clontech). Overlapping sequence was used to confirm the full length clone. The full length protein sequence of human ADAMTS-9 is shown in Figure 29. The 5′ end of the clone contains a methionine codon within a good Kozak consensus for translation initiation. A signal peptide sequence is located just downstream of this methionine in the translated ORF, and the size of the pro-domain is similar to that of other ADAM-TS family members. Therefore, this appears to be the starting methionine of ADAMTS-9.

The overall protein sequence of ADAMTS-9 is similar to that of the other ADAM-TS proteins. All of these family members have a pro-domain, metalloprotease domain, disintegrin-like domain, thrombospondin domain, spacer region, and a variable number of a thrombospondin-like submotifs at the carboxyl-terminal end of the protein (Figure 32A). Like other ADAM-TS family members, ADAMTS 9 contains an amino-terminal signal peptide sequence and lacks a transmembrane domain.

Among the 23 ADAM family members, 10 are predicted to be active proteases based on the sequence of their Zn binding catalytic sites (Black and White, *Curr. Opin. Cell. Biol 10*:654-659, 1998). The consensus catalytic sequence site based on ADAM and snake venom metalloproteases is HE*X*GH*XX*G*XX*HD (SEQ ID NO:51). The ADAM-TS family of proteins has homology to this consensus sequence except at the second conserved glycine. ADAMTS 9 has an asparagine at this conserved glycine site in the helix. Two other ADAM-TS proteins, ADAMTS-1 and ADAMTS-4, also have an asparagine in this position instead of glycine (Figure 32B). This suggests that ADAMTS-9, line ADAMTS-1 and ADAMTS-4, may have an active metalloprotease domain.

It has been proposed that an invarient cysteine residue in the pro-domain of MMP and ADAM proteins coordinates the catalytic Zn ion in the metalloprotease domain, thus maintaining the protease in an inactive state (Loechel et al., J. Biol Chem. 274:13427-33, 1999). Once the pro-domain is cleaved this interaction is interrupted and the protease is activated by a "cysteine switch" mechanism. A proposed cysteine switch

residue in ADAMTS-9 is marked in Figure 29 by a star. Proteolytic processing of the pro-domain of ADAM and ADAM-TS proteins is believed to occur by furin endopeptidases in the Golgi. ADAMTS-9 contains two potential furin cleavage sites (consensus R*X*(K/R)R; SEQ ID NO:35) at the end of the pro-domain (see Figure 29). Based on the sequence of mature murine *ADAMTS-1*, the second furin cleavage site is most likely used in ADAMTS-9 (resulting amino-terminus FLSYPR).

Following the metalloprotease domain, ADAMTS-9 contains a cysteine-rich region that has homology to the disintegrin domain in snake venom metalloprotease and ADAMs. Next, all of the ADAM-TS family members contain an internal TSP1 motif that has the two conserved heparin binding segments: W(S/G)*X*WS*X*W (SEQ ID NO:36) and CSVTCG (SEQ ID NO:37). Separating the internal TSP1 motif and the carboxy terminal TSP1-like submotifs is a variable length spacer region. As seen in Figure 32A, most ADAM-TS family members have between one and three TSP1-like submotifs at the end of the protein. However at the extremes are ADAMTS 3 which has no TSP1-like motifs and *C. elegans* GON-1 which has 17 of these motifs. ADAMTS-9 contains one internal TSP1 motif and three TSP-1 like submotifs at the carboxyl end (Figure 30A). A possible role for ADAMTS 9 in the adult is suppression of angiogenesis through the carboxy-terminal TSP1 motifs.

Overall, the predicted mature forms of the ADAM-TS proteins show 20-40% similarity to each other. Interestingly, by BLAST analysis ADAMTS-9 shows as much homology to *C. elegans* GON-1 as to other human ADAM-TS, suggesting that ADAMTS 9 may be the human homologue of GON-1. The dendrogram in Figure 30C (prepared with the MegAlign program (DNAStar)) shows the relationship between the known human ADAM-TS members, ADAMTS 9, and GON-1.

The expression pattern of ADAMTS 9 was examined in a variety of human adult and fetal tissues using RT-PCR. For tissue distribution analysis, human multiple tissue cDNA panels I and II were purchased from Clontech. RT-PCR was performed using a touchdown procedure where the annealing temperature was dropped from 63°C to 57°C over 10 cycles then kept at 57°C for 20 cycles. The sense primer was CAGGGGAAACAGACGATGACAACT (SEQ ID NO:38) and the antisense

primer was TGCGGTAACCCAAGCCACACT (SEQ ID NO:39). Expected product size was 510 bp. Control primers to glyceraldehyde-3-phosphate dehydrogenase (G3PDH) were supplied by Clontech--expected size is about 1 kb.

As seen with other ADAM-TS genes, Northern blot analysis showed very low levels of expression. Therefore a more sensitive RT-PCR procedure was used. The cDNA panels used were normalized to the mRNA expression levels of several different housekeeping genes to ensure accurate assessment of tissue specificity. ADAMTS-9 was found in ovary, pancreas, heart, kidney, lung, placenta, and strikingly in all fetal tissues examined (Figure 31), suggesting a possible role in development. In addition, using hybridization to cDNA libraries we have identified ADAMTS-9 in adult spinal cord and brain. However, ADAMTS-9 was not detected in colon, leukocyte, prostate, small intestine, testis, liver, skeletal muscle, spleen or thymus (Figure 31). Expression of the G3PDH housekeeping gene in all cDNAs tested is shown as a control for template integrity and the RT-PCR procedure. One notable difference in the expression pattern of ADAMTS-9 compared to other ADAMTS genes is the presence of ADAMTS-9 in the adult kidney. This is of interest since the chromosomal locus containing ADAMTS-9 is often deleted in renal tumors.

A genomic clone of ADAMTS 9 was obtained by screening a human P1 library and used for FISH analysis (Genome Systems). Briefly, the human ADAMTS-9 genomic clone was labeled with digoxigenin dUTP by nick translation. Labeled probe was combined with sheared human DNA and hybridized to normal metaphase chromosomes derived from PHA stimulated peripheral blood lymphocites in a solution containing 50% formamide, 10% dextran sulfate and 2X SSC. Specific hybridization signals were detected by incubating the hybridized slides in fluoresceinated antidigoxigenin antibodies followed by counterstaining with DAPI for one-color experiments. Probe detection for two-color experiments was accomplished by incubating the slides in fluoresceinated antidigoxigenin antibodies and Texas red avidin followed by counterstaining with DAPI. A total of 80 metaphase cells were analyzed with 70 exhibiting specific labeling. Initial FISH experiments resulted in specific labeling of the short arm of chromosome 3. Measurement of 10 specifically labeled

chromosome 3's demonstrated that ADAMTS-9 is located at a position which is 30% the distance from the centromere to the telomere of chromosome arm 3p, an area which corresponds to 3p14.3-21.1 (Figures 32A and 32B). Since deletions and other rearrangements of this locus are frequent and early events in the pathogenesis of a number of human cancers (including renal cell carcinoma, breast cancers, uterine cervical carcinoma and vulvar carcinomas, this region may contain one or more tumor suppressor genes.

The chromosomal localization of the human ADAMTS 9 locus was independently confirmed by PCR analysis of the Stanford G3 radiation hybrid mapping panel. The G3 hybrid mapping panel (Stewart et al., *Genomic Res.* 7:422-433, 1997) containing 83 radiation hybrid DNA, as well as human and hamster control DNAs was obtained from Research genetics Inc. (Huntsville, Alabama). The human chromosome content of each somatic cell hybrid was established by the Stanford Human Genome Center using more than 10,000 STSs derived from random genetic markers and expressed tagged sequences (http://www-shgc.stanford.edu/Mapping/rh/). PCR reactions were carried out in a 10 µl reaction volume containing 25 ng DNA template, 25 µm deoxynucleotide triphosphates, 20 pmol of each oligonucleotide primer, 0.5 U of Taq polymerase (Boehringer Mannheim), 2.5 mM $MgCl_2$, 50 mM KCl and 10 mM Tris-HCl (pH 8.3). The sense primer is GTGCGCTGGGTCCCTAAATAC (SEQ ID NO:40) which is in the coding sequence and the antisense primer is AAAATCACAGGTTGGCAGCGG (SEQ ID NO:41) which is in an intronic sequence. Thirty cycles of PCR were performed. Ten cycles consisted of denaturing at 94°C for 15 seconds, annealing at 62°C for 30 seconds, going down 0.5°C each cycle and extension at 72°C for 30 seconds. Twenty more cycles were performed using the same denaturing and extension conditions and keeping the annealing at 57°C for 30 seconds. PCR was proceeded by a 2 min incubation at 94°C and followed by a 72°C final soak for 10 minutes. Amplified products were electrophoresed through a 2% agarose gel and visualized by ethidium bromide staining. The resulting PCR product was a 302 bp human specific fragment. The presence or absence of the ADAMTS 9 product was scored for each of the somatic cell hybrids. The results were submitted to the Stanford

Radiation Hybrid Server via the internet (http://www-shgc.stanford.edu) and the completed data were returned to us. ADAMTS 9 was linked to the ordered markers SHGC-33668 with a LOD score of 11.47 and SHGC-20118 (D3S3571) with a LOD score of 11.06. The results confirm localization of ADAMTS 9 to the short arm of chromosome 3 and place ADAMTS-9 within the context of established maps. Furthermore SHGC-20118 (D3S3571) has been mapped to 3p14.2, placing ADAMTS-9 closer to the 14.2-14.3 region of chromosome 3. This location is interesting in that it contains a well characterized breakpoint for translocations common in hereditary renal cell carcinomas.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the present invention is not limited except as by the appended claims.

CLAIMS

1. An isolated polynucleotide that encodes an ADAMTS polypeptide, wherein the polypeptide comprises:

(a) at least 50 consecutive amino acid residues of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:2, 4, 10, 14, 16, 18, 22, 24, 26 or 27; or

(b) a variant of any of the foregoing amino acid sequences that differs in one or more substitutions, deletions, additions and/or insertions, wherein substitutions, if any, are present at no more than 10% of the consecutive residues of the ADAMTS protein.

2. A polynucleotide according to claim 1, wherein the polynucleotide comprises a sequence recited in any one of SEQ ID NOs:1, 3, 9, 13, 15, 17, 21, 23 or 25.

3. A polynucleotide according to claim 1, wherein substitutions, if any, are present at no more than 5% of the consecutive residues of the ADAMTS protein.

4. A polynucleotide according to claim 1, wherein the polypeptide has an ADAMTS activity that is not substantially diminished relative to the ADAMTS protein.

5. A recombinant expression vector comprising a polynucleotide according to claim 1.

6. A host cell transformed or transfected with an expression vector according to claim 5.

7. An isolated antisense polynucleotide complementary to at least 20 consecutive nucleotides present within a polynucleotide according to claim 1.

8. A method for preparing an ADAMTS polypeptide, the method comprising:

(a) culturing a host cell transformed or transfected with an expression vector comprising a polynucleotide that encodes an ADAMTS polypeptide comprising:

(i) at least 50 consecutive amino acid residues of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 27; or

(ii) a variant of any of the foregoing amino acid sequences that differs in one or more substitutions, deletions, additions and/or insertions, wherein substitutions, if any, are present at no more than 10% of the consecutive residues of the ADAMTS protein;

wherein the step of culturing is performed under conditions promoting expression of the polynucleotide sequence; and

(b) recovering an ADAMTS polypeptide.

9. A method for preparing an ADAMTS polypeptide, the method comprising:

(a) culturing a host cell according to claim 6 under conditions promoting expression of the polynucleotide; and

(b) recovering an ADAMTS polypeptide.

10. An isolated ADAMTS polypeptide comprising:

(a) at least 50 consecutive amino acid residues of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:2, 4, 10, 14, 16, 18, 22, 24, 26 or 27; or

(b) a variant of any of the foregoing amino acid sequences that differs in one or more substitutions, deletions, additions and/or insertions, wherein substitutions, if any, are present at no more than 10% of the consecutive residues of the ADAMTS protein.

11. An ADAMTS polypeptide according to claim 10, wherein the polypeptide has an ADAMTS activity that is not substantially diminished relative to the ADAMTS protein.

12. A polypeptide comprising an amino acid sequence recited in any one of SEQ ID NOs:2, 4, 10, 14, 16, 18, 22, 24, 26 or 27.

13. An isolated ADAMTS polypeptide comprising:

(a) at least 50 consecutive amino acid residues of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:6, 8, 12, or 20

(b) a variant of any of the foregoing amino acid sequences that differs in one or more substitutions, deletions, additions and/or insertions, wherein substitutions, if any, are present at no more than 10% of the consecutive residues of the ADAMTS protein.

14. An ADAMTS polypeptide according to claim 13, wherein the polypeptide has an ADAMTS activity that is not substantially diminished relative to the ADAMTS protein.

15. An ADAMTS polypeptide according to claim 13, wherein the polypeptide comprises at least 40 consecutive amino acid residues of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:6, 8, 12, or 20.

16. A polypeptide comprising an amino acid sequence recited in any one of SEQ ID NOs:6, 8, 12, or 20.

17. A pharmaceutical composition comprising:

(a) an ADAMTS polypeptide comprising:

(i) at least 50 consecutive amino acid residues of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 27; or

(ii) a variant of any of the foregoing amino acid sequences that differs in one or more substitutions, deletions, additions and/or insertions, wherein substitutions, if any, are present at no more than 10% of the consecutive residues of the ADAMTS protein; and

(b) a physiologically acceptable carrier.

18. A vaccine comprising:

(a) an ADAMTS polypeptide comprising:

(i) at least 50 consecutive amino acid residues of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22. 24, 26 or 27; or

(ii) a variant of any of the foregoing amino acid sequences that differs in one or more substitutions, deletions, additions and/or insertions, wherein substitutions, if any, are present at no more than 10% of the consecutive residues of the ADAMTS protein; and

(b) a non-specific immune response enhancer.

19. An isolated antibody, or antigen-binding fragment thereof, that specifically binds to an ADAMTS polypeptide that comprises a sequence recited in any one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 27.

20. A method for screening for an agent that modulates ADAMTS protein expression in a cell, comprising:

(a) contacting a candidate modulator with a cell expressing an ADAMTS polypeptide, wherein the polypeptide comprises:

(i) at least 50 consecutive amino acid residues of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:2, 4, 6, 8, 10, 12. 14, 16, 18, 20, 22, 24, 26 or 27; or

(ii) a variant of any of the foregoing amino acid sequences that differs in one or more substitutions, deletions, additions and/or insertions, wherein

substitutions, if any, are present at no more than 10% of the consecutive residues of the ADAMTS protein; and

(b) subsequently evaluating the effect of the candidate modulator on expression of an ADAMTS mRNA or polypeptide, and therefrom identifying an agent that modulates ADAMTS protein expression in the cell.

21. A method for screening for an agent that modulates an ADAMTS protein activity, comprising:

(a) contacting a candidate modulator with an ADAMTS polypeptide, comprising:

(i) at least 50 consecutive amino acid residues of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 27; or

(ii) a variant of any of the foregoing amino acid sequences that differs in one or more substitutions, deletions, additions and/or insertions, wherein substitutions, if any, are present at no more than 10% of the consecutive residues of the ADAMTS protein;

wherein the polypeptide has an ADAMTS activity that is not substantially diminished relative to the ADAMTS protein;

and wherein the step of contacting is carried out under conditions and for a time sufficient to allow the candidate modulator to interact with the polypeptide; and

(b) subsequently evaluating the effect of the candidate modulator on an ADAMTS activity of the polypeptide, and therefrom identifying an agent that modulates an activity of an ADAMTS protein.

22. An agent that decreases expression or activity of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 27, for use in the manufacture of a medicament for inhibiting neuroinflammation in a patient.

23. An agent according to claim 22, wherein ADAMTS activity is decreased by inhibiting expression of an endogenous ADAMTS gene.

24. An agent according to claim 22, wherein ADAMTS activity is decreased by administering a modulating agent that binds to an ADAMTS protein.

25. An agent that decreases expression or activity of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 27, for use in the manufacture of a medicament for inhibiting neurodegeneration in a patient.

26. An agent according to claim 25, wherein ADAMTS activity is decreased by inhibiting expression of an endogenous ADAMTS gene.

27. An agent according to claim 25, wherein ADAMTS activity is decreased by administering a modulating agent that binds to an ADAMTS protein.

28. A pharmaceutical composition according to claim 17, for use in the manufacture of a medicament for method for treating a patient afflicted with a condition associated with neuroinflammation and/or neurodegeneration.

29. A composition according to claim 28, wherein the condition is selected from the group consisting of Alzheimer's disease, Parkinson's disease and stroke.

30. A method for modulating ADAMTS activity in a cell, comprising contacting a cell expressing an ADAMTS polypeptide with an effective amount of an agent that modulates ADAMTS protein activity or expression, wherein the ADAMTS polypeptide comprises:

(i) at least 50 consecutive amino acid residues of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 27; or

(ii) a variant of any of the foregoing amino acid sequences that differs in one or more substitutions, deletions, additions and/or insertions, wherein substitutions, if any, are present at no more than 10% of the consecutive residues of the ADAMTS protein;

wherein the polypeptide has an ADAMTS activity that is not substantially diminished relative to the ADAMTS protein;

and thereby modulating ADAMTS activity in the cell.

31. A pharmaceutical composition according to claim 17, for use in the manufacture of a medicament for treating a patient afflicted with a condition associated with cell proliferation, cell migration, inflammation and/or angiogenesis.

32. A composition according to claim 31, wherein the condition is selected from the group consisting of cancer, arthritis and autoimmune diseases.

33. An agent that decreases expression or activity of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 27, for use in the manufacture of a medicament for treating a patient afflicted with an invasive tumor.

34. An agent that decreases expression or activity of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 27, for use in the manufacture of a medicament for treating a patient afflicted with a brain tumor.

35. An agent that decreases expression or activity of an ADAMTS protein that comprises a sequence recited in any one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20,

22, 24, 26 or 27, for use in the manufacture of a medicament for treating a patient afflicted with a brain injury.

36. An agent according to any one of claims 33-35, wherein the ADAMTS protein comprises a sequence recited in SEQ ID NO:16.

AGGACCAAGCGGTTTGTGTCTGAGGCGCGCTTCGTGGAGACGCTGCTGGTGGCCGATGCGTCCATGGCTGCCTTCTACGG
GGCCGACCTGCAGAACCACATCCTGACGTTAATGTCTGTGGCAGCCCGAATCTACAAGCACCCCAGCATCAAGAATTCCA
TCAACCTGATGGTGGTAAAAGTGCTGATCGTAGAAGATGAAAAATGGGGCCCAGAGGTGTCCGACAATGGGGGGCTTACA
CTGCGTAACTTCTGCAACTGGCAGCGGCGTTTCAACCAGCCCAGCGACCGGCACCCAGAGCACTACGACACGGCCATCCT
GCTCACCAGACAGAACTTCTGTGGGCAGGAGGGGCTGTGTGACACCCTGGGTGTGGCAGACATCGGGACCATTTGTGACC
CCAACAAAAGCTGCTCCGTGATCGAGGATGAGGGGCTCCAGGCGGCCCACACCCTGGCCCATGAACTAGGGCACGTCCTC
AGCATGCCCCACGACGACTCCAAGCCCTGCACACGGCTCTTCGGGCCCATGGGCAAGCACCACGTGATGGCACCGCTGTT
CGTCCACCTGAACCAGACGCTGCCCTGGTCCCCCTGCAGCGCCATGTATCTCACAGAGCTTCTGGACGGCGGGCACGGAG
ACTGTCTCCTGGATGCCCCTGCTGCGGCCCTGCCCCTCCCCACAGGCCTCCCGGGCCGCATGGCCCTGTACCAGCTGGAC
CAGCAGTGCAGGCAGATCTTTGGGCCGGATTTCCGCCACTGCCCCAACACCTCTGCTCAGGACGTCTGCGCCCAGCTTTG
GTGCCACACTGATGGGGCTGAGCCCCTGTGCCACACGAAGAATGGCAGCCTGCCCTGGGCTGACGGCACGCCGTGCGGGC
CTGGGCACCTCTGCTCAGAAGGCAGCTGTCTACCTGAGGAGGAAGTGGAGAGGCCCAAGCCCGTGGTAGATGGAGGCTGG
GCACCGTGGGGACCCTGGGGAGAATGTTCTCGGACCTGTGGAGGAGGAGTACAGTTTTCACACCGTGAGTGCAAGGACCC
CGAGCCTCAGAATGGAGGAAGATACTGCCTGGGTCGGAGAGCCAAGTACCAGTCATGCCACACGGAGGAATGCCCCCCTG
ACGGGAAAAGCTTCAGGGAGCAGCAGTGTGAGAAGTATAATGCCTACAATTACACTGACATGGACGGGAATCTCCTGCAG
TGGGTCCCCAAGTATGCTGGGGTGTCCCCCCGGGACCGCTGCAAGTTGTTCTGCCGAGCCCGGGGGAGGAGCGAGTTCAA
AGTGTTCGAGGCCAAGGTGATTGATGGCACCCTGTGTGGGCCAGAAACACTGGCCATCTGTGTCCGTGGCCAGTGTGTCA
AGGCCGGCTGTGACCATGTGGTGGACTCGTTTTGGAAGCTGGACAAATGCGGGGTGTGTGGGGGGAAAGGCAACTCCTGC
AGGAAGGGCTCCGGGTCCCTCACCCCCACCAATTATGGCTACAATGACATTGTCACCATCCCAGCTGGTGCCACTAATAT
TGACGTGAAGCAGCGGAGCCACCCGGGTGTGCAGAACGATGGGAACTACCTGGCGCTGAAGACGGCTGATGGGCAGTACC
TGCTCAACGGCAACCTGGCCATCTCTGCCATAGAGCAGGACATCTTGGTGAAGGGGACCATCCTGAAGTACAGCGGCTCC
ATCGCCACCCTGGAGCGCCTGCAGAGCTTCCGGCCCTTGCCAGAGCCTCTGACAGTGCAGCTCCTGGCAGTCCCTGGCGA
GGTCTTCCCCCCAAAAGTCAAATACACCTTCTTTGTTCCTAATGACGTGGACTTTAGCATGCAGAGCAGCAAAGAGAGAG
CAACCACCAACATCACCCAGCCGCTGCTCCACGCACAGTGGGTGCTGGGGGACTGGTCTGAGTGCTCTAGCACCTGCGGG
GCCGGCTGGCAGAGGCGAACTGTAGAGTGCAGGGACCCCTCCGGCCAGGCCTCTGCCACCTGCAACAAGGCTCTGAAACC
CGAGGATGCCAAGCCCTGCGAAAGCCAGCTGTGCCCCCTGTGATTCAGGGGGGCAGGGGCCAGTCTTGTGCTCCTGGACA
TGCGGTACTGAGGTGCAGACAAGGGTCTCCACTGTGGTGACTGGGTCCCTTGGCCATATCAAGGCAGCACGGCCCACCCA
GGCCTCCCATTGCCGCAACCCCTCCAGTACTGCACAAATTCCTAAGGGGGAAGAGGAGAGGGTATGGGGCGGCAGACCCT
ATCATCAACTGTCCAGTGGACTGGACCTTGCTCGGGTTCAAGTAGAGGGCATAGGTTAAAAGGTAAAAGTGCACTTATTG
TACCAGACAGGACGCCCGCGAATTC

Fig. 1

RTKRFVSEARFVETLLVADASMAAFYGADLQNHILTLMSVAARIYKHPSIKNSINLMVVKVLIVEDEKWGPEVSDNGGLT
LRNFCNWQRRFNQPSDRHPEHYDTAILLTRQNFCGQEGLCDTLGVADIGTICDPNKSCSVIEDEGLQAAHTLAHELGHVL
SMPHDDSKPCTRLFGPMGKHHVMAPLFVHLNQTLPWSPCSAMYLTELLDGGHGDCLLDAPAAALPLPTGLPGRMALYQLD
QQCRQIFGPDFRHCPNTSAQDVCAQLWCHTDGAEPLCHTKNGSLPWADGTPCGPGHLCSEGSCLPEEEVERPKPVVDGGW
APWGPWGECSRTCGGGVQFSHRECKDPEPQNGGRYCLGRRAKYQSCHTEECPPDGKSFREQQCEKYNAYNYTDMDGNLLQ
WVPKYAGVSPRDRCKLFCRARGRSEFKVFEAKVIDGTLCGPETLAICVRGQCVKAGCDHVVDSFWKLDKCGVCGGKGNSC
RKGSGSLTPTNYGYNDIVTIPAGATNIDVKQRSHPGVQNDGNYLALKTADGQYLLNGNLAISAIEQDILVKGTILKYSGS
IATLERLQSFRPLPEPLTVQLLAVPGEVFPPKVKYTFFVPNDVDFSMQSSKERATTNITQPLLHAQWVLGDWSECSSTCG
AGWQRRTVECRDPSGQASATCNKALKPEDAKPCESQLCPL.

Fig. 2

CCCCCCCTCGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCCGGGCCCCCCACCCCCGCCCCTGAAACTTCTATAG
CAAATAGCAAACATCCAGCTAGACTCAGTCGCGCAGCCCCTCCCGGCGGGCAGCGCACTATGCGGCTCGAGTGGGCGTCC
TTGCTGCTGCTACTGCTGCTGCTGTGCGCGTCCTGCCTGGCCCTGGCCGCTGACAACCCTGCCGCGGCACCTGCCCAGGA
TAAAACCAGGCAGCCTCGGGCTGCTGCAGCGGCTGCCCAGCCCGACCAGCGGCAGTGGGAGGAAACACAGGAGCGGGGCC
ATCTGCAACCCTTGGCCAGGCAGCGCAGGAGCAGCGGGCTGGTGCAGAATATAGACCAACTCTACTCTGGCGGTGGCAAA
GTGGGCTACCTTGTCTACGCGGGCGGCCGGAGGTTCCTGCTGGACCTGGAGAGGGATGACACAGTGGGTGCTGCTGGTGG
CATCGTTACTGCAGGAGGGCTGAGCGCATCCTCTGGCCACAGGGGTCACTGCTTCTACAGAGGCACTGTGGACGGCAGCC
CTCGATCCCTAGCTGTCTTTGACCTCTGTGGGGGTCTCGATGGCTTCTTCGCAGTCAAGCATGCGCGCTACACTCTGAGG
CCGCTCTTGCGTGGGTCCTGGGCAGAGTCCGAACGAGTTTACGGGGATGGGTCTTCACGCATCCTGCATGTCTACACCCG
CGAGGGCTTCAGCTTCGAGGCCCTGCCGCCACGCACCAGTTGCGAGACTCCAGCGTCCCCGTCTGGGGCCCAAGAGAGCC
CCTCGGTGCACAGTAGTTCTAGGCGACGCACAGAACTGGCACCGCAGCTGCTGGACCATTCAGCTTTCTCGCCAGCTGGG
AACGCGGGACCTCAGACCTGGTGGAGGCGGAGGCGCCGTTCCATCTCCAGGGCCCGCCAGGTGGAGCTCCTCTTGGTGGC
TGACTCTTCCATGGCCAAGATGTATGGGCGGGGCCTGCAGCATTACCTGCTGACCCTGGCCTCTATTGCCAACCGGCTGT
ACAGTCATGCAAGCATCGAGAACCACATCCGCCTGGCCGTAGTGAAAGTGGTGGTGCTGACCGACAAGAGTCTGGAGGTG
AGCAAGAACGCGGCCACGACCCTCAAGAACTTTTGCAAATGGCAGCACCAACACAACCAGCTAGGTGATGACCATGAGGA
GCACTACGATGCAGCCATCCTGTTCACCAGAGAGGATTTATGTGGGCATCATTCATGTGACACCCTGGGAATGGCAGACG
TTGGGACCATATGTTCTCCGGAGCGCAGCTGCGCTGTGATTGAAGATGATGGCCTCCATGCAGCTTTCACTGTGGCTCAC
GAAATTGGACATCTACTTGGCCTCTCTCACGACGATTCCAAATTCTGTGAAGAGAACTTTGGTTCTACAGAAGACAAGCG
TTTAATGTCTTCAATCCTTACCAGCATTGATGCATCCAAGCCCTGGTCCAAATGCACTTCAGCCACGATCACAGAATTTC
TGGATGACGGTCATGGTAACTGTTTACTAGATGTACCACGGAAGCAGATTCTGGGCCCCGAGGAACTCCCAGGACAGACC
TATGATGCCACCCAGCAGTGCAACTTGACATTTGGGCCTGAATACTCTGTGTGCCCTGGCATGGATGTCTGTGCACGGCT
GTGGTGTGCTGTGGTGCGCCAAGGCCAAATGGTGTGTCTGACCAAGAAGTTGCCTGCCGTGGAGGGCACTCCCTGTGGGA
AAGGAAGAATCTGCCTGCAAGGCAAATGTGTGGACAAAACTAAGAAAAAATATTACTCGACATCAAGCCATGGAAATTGG
GGGTCCTGGGGCCCCTGGGGTCAGTGTTCTCGCTCTTGCGGGGGAGGAGTACAGTTTGCCTACCGCCATTGCAATAACCC
CGCACCTCGAAACAGTGGCCGCTACTGCACAGGGAAGAGGGCCATATACCGTTCCTGCAGTGTCATACCCTGCCCACCTA
ACGGCAAATCTTTCCGCCACGAGCAGTGTGAAGCCAAAAATGGCTATCAGTCCGATGCAAAAGGAGTCAAAACATTTGTA
GAATGGGTTCCCAAATACGCAGGTGTCCTGCCGGCAGACGTGTGCAAGCTTACGTGCAGAGCTAAGGGCACTGGCTATTA
CGTGGTCTTTTCTCCAAAGGTTACAGATGGGACAGAATGTAGACCCTACAGCAACTCCGTGTGTGTCCGAGGGAGGTGCG
TGAGAACGGGGTGTGACGGCATCATCGGCTCAAAGCTACAGTATGACAAGTGTGGAGTGTGTGGAGGGGATAACTCCAGT

Fig. 3A

TGTACAAAGATTATCGGAACCTTCAATAAAAAAAGCAAGGGTTATACTGACGTTGTGAGGATCCCTGAAGGAGCAACCCA
CATAAAAGTCCGACAGTTCAAAGCCMAAGACCAGACTAGATTCACTGCTTACTTAGCCCTAAAGAAGAAAACTGGCGAGT
ACCTTATCAACGGCAAGTACATGATCTCCACTTCAGAGACCATCATCGACATCAATGGTACCGTCATGAACTACAGTGGG
TGGAGTCACAGAGATGATTTTTTTACATGGGATGGGCTATTCAGCCACAAAGGAAATTCTGATTGTGCAGATCCTTGCAAC
AGACCCAACTAAAGCATTAGACGTCCGTTACAGCTTTTTTTGTTCCCAAGAAGACCACTCAAAAAGTGAATTCCTGCAGCC
CGGGGGATCCACTAGTTCTAGAGCGGCCG

Fig. 3B

MRLEWASLLLLLLLLCASCLALAADNPAAAPAQDKTRQPRAAAAAAQPDQRQWEETQERGHLQPLARQRRSSGLVQNIDQ
LYSGGGKVGYLVYAGGRRFLLDLERDDTVGAAGGIVTAGGLSASSGHRGHCFYRGTVDGSPRSLAVFDLCGGLDGFFAVK
HARYTLRPLLRGSWAESERVYGDGSSRILHVYTREGFSFEALPPRTSCETPASPSGAQESPSVHSSSRRRTELAPQLLDH
SAFSPAGNAGPQTWWRRRRRSISRARQVELLLVADSSMAKMYGRGLQHYLLTLASIANRLYSHASIENHIRLAVVKVVVL
TDKSLEVSKNAATTLKNFCKWQHQHNQLGDDHEEHYDAAILFTREDLCGHHSCDTLGMADVGTICSPERSCAVIEDDGLH
AAFTVAHEIGHLLGLSHDDSKFCEENFGSTEDKRLMSSILTSIDASKPWSKCTSATITEFLDDGHGNCLLDVPRKQILGP
EELPGQTYDATQQCNLTFGPEYSVCPGMDVCARLWCAVVRQGQMVCLTKKLPAVEGTPCGKGRICLQGKCVDKTKKKYYS
TSSHGNWGSWGPWGQCSRSCGGGVQFAYRHCNNPAPRNSGRYCTGKRAIYRSCSVIPCPPNGKSFRHEQCEAKNGYQSDA
KGVKTFVEWVPKYAGVLPADVCKLTCRAKGTGYYVVFSPKVTDGTECRPYSNSVCVRGRCVRTGCDGIIGSKLQYDKCGV
CGGDNSSCTKIIGTFNKKSKGYTDVVRIPEGATHIKVRQFKAXDQTRFTAYLALKKKTGEYLINGKYMISTSETIIDING
TVMNYSGWSHRDDFLHGMGYSATKEILIVQILATDPTKALDVRYSFFVPKKTTQKVNSCSPGDPLVLERP

Fig. 4

KIAA0605 Accession #: AB011177

```
cactggcgga gaaaatcccc ttctttttt tctctctctt tttttctttt tgagacggaa     60
tctcactctt tcacccagac tggagggcag cggcgagatc tcggctcact gcaacctcca    120
cctcccaggt tcaagcaatt ctcctgcctc agccttccga gtagctggga ttacaggtgc    180
ccgccaccac gcccagctaa ttttttgtatt tttagtagag acaggatttt accatgttgg    240
ccatgctggt ctcaaactcc tgacctcgtg tgatcccccc gccttcagcct ctcaaactgc    300
tgggattata ggcatgagcc actgcgcctg gccaacaatc cccttctaaa ggcaggtggt    360
gtctccagca ccagggccat acggctgcaa caccccctaca agtgccgggt ctgccagaca    420
accacgacca actagtccca gataaccttg aggcctgggc actggctggg ccccgagggc    480
tcttcccaaa gcgtaccctg gtcatctgga agaggatcgg agctggcctg gtggtgacag    540
tggcctgcct tcctaggatg gatggcagat ggcaatgttc ctgctgggcc tggttcctgc    600
tggttctggc agttgtagct ggggacacag tgtcaaccgg gtccacggac aacagcccaa    660
catccaatag cctggagggg ggcaccgacg ccacggcctt ctggtggggg gagtggacca    720
agtggacggc gttttccccc agttgcgggg gtggggtgac atcccaggag cggcactgcc    780
tgcagcagag gaggaagtcc gtcccgggcc ccgggaacag gacctgcacg ggcacgtcca    840
agcggtacca gctctgcaga gtgcaggagt gtccgccgga cgggaggagc ttccgcgagg    900
agcagtgcgt ctccttcaac tcccacgtgt acaacgggcg gacgcaccag tggaagcctc    960
tgtacccgga tgactatgtc cacatctcca gcaaaccgtg tgacctgcac tgtaccaccg   1020
tggacggcca gcggcagctc atggtccccg cccgcgacgg cacatcctgc aagctcactg   1080
acctgcgagg ggtttgcgtg tctggaaaat gtgagcccat cggctgtgac ggggtgcttt   1140
tctccaccca cacactggac aagtgtggca tctgccaggg ggacggtagc agctgcaccc   1200
acgtgacggg caactatcgc aaggggaatg cccaccttgg ttactctctg gtgacccaca   1260
tcccggctgg tgcccgagac atccagattg tagagaggaa gaagtccgct gacgtgctag   1320
ctcttgcaga tgaagctggc tactacttct tcaacggcaa ctacaaggtg gacagcccca   1380
agaacttcaa catcgctggc acggtggtca agtaccggcg gcccatggat gtctatgaga   1440
ccggaatcga gtacatcgtg gcacaggggc ccaccaacca gggcctgaat gtcatggtgt   1500
ggaaccagaa cggcaaaagc ccctccatca ccttcgagta cacgctgctg cagccgccac   1560
acgagagccg cccccagccc atctactatg gcttctccga gagcgctgag agccagggcc   1620
tggacggggc cgggctgatg ggcttcatcc cgcacaacgg ctccctctac ggccaggcct   1680
cctcagagcg gctgggcctg gacaaccggc tgttcggcca cccgggcctg gacatggagc   1740
tgggccccag ccagggccag gagaccaacg aggtgtgcga gcaggccggc ggcggggcct   1800
gcgaggggcc ccccaggggc aagggcttcc gagaccgcaa cgtcacgggg actcctctca   1860
ccggggacaa ggatgacgaa gaggttgaca cccacttcgc ctcccaggag ttcttctcgg   1920
ctaacgccat ctctgaccag ctgctgggcg caggctctga cttgaaggac ttcaccctca   1980
atgagactgt gaacagcatc tttgcacagg gcgccccaag gagctccctg gccgagagct   2040
tcttcgtgga ttatgaggag aacgaggggg ctggccctta cctgctcaac gggtcctacc   2100
tggagctgag cagcgacagg gttgccaaca gctcctccga ggccccattc cccaacgtta   2160
gcaccagcct gctcacctcg gccgggaaca ggactcacaa ggccaggacc aggcccaagg   2220
cgcgcaagca aggcgtgagt cccgcggaca tgtaccggtg gaagctctcg tcccacgagc   2280
cctgcagtgc cacctgcacc acaggggtca tgtctgcgta cgccatgtgt gtccgctatg   2340
atggcgtcga ggtggatgac agctactgtg acgccctgac ccgtcccgag cctgtccacg   2400
agttctgcgc tgggagggag tgccagccca ggtgggagac gagcagctgg agcgagtgtt   2460
```

Fig. 5A

cgcgcacctg cggagagggc taccagttcc gcgtcgtgcg ctgctggaag atgcctctgc 2520
ccggcttcga cagctccgtg tacagcgacc tgtgcgaggc agccgaggcc gtgcggcccg 2580
aggaacgcaa gacctgccgg aaccccgcct gcgggcccca gtgggagatg tcggagtggt 2640
ccgagtgcac tgccaagtgt ggggagcgca gtgtggtgac cagggacatc cgctgctcgg 2700
aggatgagaa gctgtgtgac cccaacacca ggcctgtagg ggagaagaac tgcacgggcc 2760
cgccctgtga ccggcagtgg accgtctccg actggggacc gtgcagtgga agctgcgggc 2820
aaggccgcac catcaggcac gtgtactgca agaccagcga cggacgggta gtacctgagt 2880
cccagtgcca gatggagacc aagcctctgg ccatccaccc ctgtggggac aaaaactgtc 2940
ccgcccactg gctggcccag gactgggagc ggtgcaacac cacctgcggg cgcggggtca 3000
agaagcggct ggtgctctgc atggagctgg ccaacgggaa gccgcagacg cgcagtggcc 3060
ccgagtgcgg gctcgccaag aagcctcccg aggagagcac gtgtttcgag aggccctgct 3120
tcaagtggta caccagcccc tggtcagagt gcaccaagac ctgcggggtg ggcgtgagga 3180
tgcgagacgt caagtgctac caggggaccg acatcgtccg tggttgcgat ccgttggtga 3240
agcccgttgg cagacaggcc tgtgatctgc agccctgccc cacggagccc ccagatgaca 3300
gctgccagga ccagccaggc accaactgtg ccctggccat caaagtgaac ctctgcgggc 3360
actggtacta cagcaaggcg tgctgccgct cctgcaggcc cccccactcc taggcccggc 3420
agctgcagcc cctccagat gaagaccaag cgcccctcct ggggctgctg cagcttctgg 3480
ggcctccaca gacccccctc ctgcggggca cgctggccta agagacgtgg cactgagcct 3540
cggctgtcga gaggggactt cccacggccc gtggaccttt gtgctcctgg ggcagagcct 3600
ccggcaccca gtggcctccc ccagacagag ccacccctgc cgtgggaacc tgtccgtgtt 3660
cctgcgtgga tcctgtgttt gtggctccca ctccccagcc ccccagcagc ccccagccga 3720
ggggcccagg gcccacagcc agcggtggag gtgtcttgct ccgggcccgt agcccacgcc 3780
ctctctgggt ggcagggcct tctgaaggaa acttgcaggc gagcccaacg tggtgggggg 3840
ccttcctccc tcagaggcca tggggtgaga ggggctcagg cagccaagga ggcccaggcg 3900
tgctccctct tatggagccc ctcccatgga gctctcttcc cgccgcactt tctaccccgg 3960
gcagaggcgc ttgcccacgg gacgtttggg gatggacctc ggcccccgcc cctgcagtca 4020
gcgtcagtgc tcatctacgt taataaagtg gtcctattta tggcggc 4067

Fig. 5B

MDGRWQCSCWAWFLLVLAVVAGDTVSTGSTDNSPTSNSLEGGTDATAFWWGEWTKWTAFSRSCGGGVTSQERHCLQQRRKSVPGPGNRTCTGTSKRYQ
LCRVQECPPDGRSFREEQCVSFNSHVYNGRTHQWKPLYPDDYVHISSKPCDLHCTTVDGQRQLMVPARDGTSCKLTDLRGVCVSGKCEPIGCDGVLFS
THTLDKCGICQGDGSSCTHVTGNYRKGNAHLGYSLVTHIPAGARDIQIVERKKSADVLALADEAGYYFFNGNYKVDSPKNFNIAGTVVKYRRPMDVYE
TGIEYIVAQGPTNQGLNVMVWNQNGKSPSITFEYTLLQPPHESRPQPIYYGFSESAESQGLDGAGLMGFIPHNGSLYGQASSERLGLDNRLFGHPGLD
MELGPSQGQETNEVCEQAGGGACEGPPRGKGFRDRNVTGTPLTGDKDDEEVDTHFASQEFFSANAISDQLLGAGSDLKDFTLNETVNSIFAQGAPRSS
LAESFFVDYEENEGAGPYLLNGSYLELSSDRVANSSSEAPFPNVSTSLLTSAGNRTHKARTRPKARKQGVSPADMYRWKLSSHEPCSATCTTGVMSAY
AMCVRYDGVEVDDSYCDALTRPEPVHEFCAGRECQPRWETSSWSECSRTCGEGYQFRVVRCWKMLSPGFDSSVYSDLCEAAEAVRPEERKTCRNPACG
PQWEMSEWSECTAKCGERSVVTRDIRCSEDEKLCDPNTRPVGEKNCTGPPCDRQWTVSDWGPCSGSCGQGRTIRHVYCKTSDGRVVPESQCQMETKPL
AIHPCGDKNCPAHWLAQDWERCNTTCGRGVKKRLVLCMELANGKPQTRSGPECGLAKKPPEESTCFERPCFKWYTSPWSECTKTCGVGVRMRDVKCYQ
GTDIVRGCDPLVKPVGRQACDLQPCPTEPPDDSCQDQPGTNCALAIKVNLCGHWYYSKACCRSCRPPHS (951 amino acids)

Fig. 6

DNA sequence of metalloproteinase gene (KIAA0366) Accession #: AB002364

```
gtcactttgg ttgatagcag ccgctctggt agaggttagg acttcagctg atggacaagc     60
tggtaatgaa gaaatggtgc aaatagattt accaataaag agatatagag agtatgagct    120
ggtgactcca gtcagcacaa atctagaagg acgctatctc tcccatactc tttctgcgag    180
tcacaaaaag aggtcagcga gggacgtgtc ttccaaccct gagcagttgt tctttaacat    240
cacggcattt ggaaaagatt ttcatctgcg actaaagccc aacactcaac tagtagctcc    300
tggggctgtt gtggagtggc atgagacatc tctggtgcct gggaatataa ccgatcccat    360
taacaaccat caaccaggaa gtgctacgta tagaatccgg aaaacagagc ctttgcagac    420
taactgtgct tatgttggtg acatcgtgga cattccagga acctctgttg ccatcagcaa    480
ctgtgatggt ctggctggaa tgataaaaag tgataatgaa gagtatttca ttgaaccctt    540
ggaaagaggt aaacagatgg aggaagaaaa aggaaggatt catgttgtct acaagagatc    600
agctgtagaa caggctccca tagacatgtc caaagacttc cactacagag agtcggacct    660
ggaaggcctt gatgatctag gtactgttta tggcaacatc caccagcagc tgaatgaaac    720
aatgagacgc cgcagacacg cgggagaaaa cgattacaat atcgaggtac tgctgggagt    780
ggatgactct gtggtccgtt tccatggcaa agagcacgtc caaaactacc tcctgaccct    840
aatgaacatt gtgaatgaaa tttaccatga tgagtccctc ggagtgcata taaatgtggt    900
cctggtgcgc atgataatgc tgggatatgc aaagtccatc agcctcatag aaaggggaaa    960
cccatccaga agcttggaga atgtgtgtcg ctgggcgtcc caacagcaaa gatctgatct   1020
caaccactct gaacaccatg accatgcaat ttttttaacc aggcaagact ttggacctgc   1080
tggaatgcaa ggatatgctc cagtcaccgg catgtgtcat ccagtgagaa gttgtaccct   1140
gaatcatgag gatggttttt catctgcttt tgtagtagcc catgaaacgg gccatgtgtt   1200
gggaatggag catgatggac aaggcaacag gtgtggtgat gagactgcta tgggaagtgt   1260
catggctccc ttggtacaag cagcattcca tcgttaccac tggtcccgat gcagtggtca   1320
agaactgaaa agatatatcc attcctatga ctgtctcctt gatgaccctt ttgatcatga   1380
ttggcctaaa ctcccagaac ttcctggaat caattattct atggatgagc aatgtcgttt   1440
tgattttggt gttggctata aaatgtgcac cgcgttccga acctttgacc catgtaaaca   1500
gctgtggtgt agccatcctg ataatcccta cttttgtaag actaaaaagg gacctccact   1560
tgatgggact gaatgtgctg ctggaaaatg gtgctataag ggtcattgca tgtggaagaa   1620
tgctaatcag caaaaacaag atggcaattg ggggtcatgg actaaatttg gctcctgttc   1680
tcggacatgt ggaactggtg ttcgtttcag aacacgccag tgcaataatc ccatgcccat   1740
caatggtggt caggattgtc ctggtgttaa ttttgagtac cagctttgta acacagaaga   1800
atgccaaaaa cactttgagg acttcagagc acagcagtgt cagcagcgaa actcccactt   1860
tgaataccag aataccaaac accactggtt gccatatgaa catcctgacc ccaagaaaag   1920
atgccacctt tactgtcagt ccaaggagac tggagatgtt gcttacatga aacaactggt   1980
gcatgatgga acgcactgtt cttacaaaga tccatatagc atatgtgtgc gaggagagtg   2040
tgtgaaagtg ggctgtgata aagaaattgg ttctaataag gttgaggata agtgtggtgt   2100
ctgtggagga gataattccc actgccgaac cgtgaagggg acatttacca gaactcccag   2160
gaagcttggg taccttaaga tgtttgatat acccccctggg gctagacatg tgttaatcca   2220
agaagacgag gcttctcctc atattcttgc tattaagaac caggctacag gccattatat   2280
tttaaatggc aaaggggagg aagccaagtc gcggaccttc atagatcttg gtgtggagtg   2340
ggattataac attgaagatg acattgaaag tcttcacacc gatggacctt tacatgatcc   2400
tgttattgtt ttgattatac ctcaagaaaa tgatacccgc tctagcctga catataagta   2460
catcatccat gaagactctg tacctacaat caacagcaac aatgtcatcc aggaagaatt   2520
```

Fig. 7A

agatactttt gagtgggctt tgaagagctg gtctcaggtt tccaaaccct gtggtggagg 2580
tttccagtac actaaatatg gatgccgtag gaaaagtgat aataaaatgg tccatcgcag 2640
cttctgtgag gccaacaaaa agccgaaacc tattagacga atgtgcaata ttcaagagtg 2700
tacacatcca ctctgggtag cagaagaatg ggaacactgc accaaaacct gtggaagttc 2760
tggctatcag cttcgcactg tacgctgcct tcagccactc cttgatggca ccaaccgctc 2820
tgtgcacagc aaatactgca tgggtgaccg tcccgagagc cgccggccct gtaacagagt 2880
gccctgccct gcacagtgga aaacaggacc ctggagtgag tgttcagtga cctgcggtga 2940
aggaacggag gtgaggcagg tcctctgcag ggctggggac cactgtgatg gtgaaaagcc 3000
tgagtcggtc agagcctgtc aactgcctcc ttgtaatgat gaaccatgtt tgggagacaa 3060
gtccatattc tgtcaaatgg aagtgttggc acgatactgc tccataccag gttataacaa 3120
gttatgttgt gagtcctgca gcaagcgcag tagcaccctg ccaccaccat accttctaga 3180
agctgctgaa actcatgatg atgtcatctc taaccctagt gacctcccta gatctctagt 3240
gatgcctaca tctttggttc cttatcattc agagacccct gcaaagaaga tgtctttgag 3300
tagcatctct tcagtgggag gtccaaatgc atatgctgct ttcaggccaa acagtaaacc 3360
tgatggtgct aatttacgcc agaggagtgc tcagcaagca ggaagtaaga ctgtgagact 3420
ggtcaccgta ccatcctccc cacccaccaa gagggtccac ctcagttcag cttcacaaat 3480
ggctgctgct tccttctttg cagccagtga ttcaataggt gcttcttctc aggcaagaac 3540
ctcaaagaaa gatggaaaga tcattgacaa cagacgtccg acaagatcat ccaccttaga 3600
aagatgagaa agtgaaccaa aaaggctaga aaccagagga aaacctggac aacctctctc 3660
ttcccatggt gcatatgctt gtttaaagtg gaaatctcta tagatcgtca gctcatttta 3720
tctgtaattg gaagaacaga aagtgctggc tcactttcta gttgctttca tcctcctttt 3780
gttctgcatt gactcattta ccagaattca ttggaagaaa tcaccaaaga ttattacaaa 3840
agaaaaatat gttgctaaga ttgtgttggt cgctctctga agcagaaaag ggactggaac 3900
caattgtgca tatcagctga ctttttgttt gttttagaaa agttacagta aaaattaaaa 3960
agagatacca atggtttaca ctttaacaag aaattttgga tatggaacaa agaattctta 4020
gacttgtatt cctatttatc tatattagaa atattgtatg agcaaatttg cagctgttgt 4080
gtaaatactg tatattgcaa aaatcagtat tattttaaga gatgtgttct caaatgattg 4140
tttactatat tacatttctg gatgttctag gtgcctgtcg ttgagtattg ccttgtttga 4200
cattctatag gttaattttc aaagcagagt attacaaaag agaagttaga attacagcta 4260
ctgacaatat aaagggtttt gttgaatcaa caatgtgata cgtaaattat agaaaaagaa 4320
aagaaacaca aaagctatag atatacagat atcagcttac ctattgcctt ctatacttat 4380
aatttaaagg attggtgtct tagtacactt gtggtcacag ggatcaacga atagtaaata 4440
atgaactcgt gcaagacaaa actgaaaccc tctttccagg acctcagtag gcaccgttga 4500
ggtgtccttt gtttttgtgt gtgtgtgttc ttttttaatt ttcgcattgt tgacagatac 4560
aaacagttat actcaatgta ctgtaataat cgcaaaggaa aaagttttgg gataacttat 4620
ttgtatgttg gtagctgaga aaaatatcat cagtctagaa ttgatatttg agtatagtag 4680
agctttgggg ctttgaaggc aggttcaaga aagcatatgt cgatggttga gatatttatt 4740
ttccatatgg ttcatgttca aatgttcaca accacaatgc atctgactgc aataatgtgc 4800
taataattta tgtcagtagt caccttgctc acagcaaagc cagaaatgct ctctccaggg 4860
agtagatgta aagtacttgt acatagaatt cagaactgaa gatatttatt aaaagttgat 4920
tttttttttct tgatagtatt tttatgtact aaatatttac actaatatca attacatatt 4980
ttggtaaact agagagacat aattagagat gcatgctttg ttctgtgcat agagaccttt 5040
aagcaaacta ctacagccaa ctcaaaagct aaaactgaac aaatttgatg ttatgcaaac 5100
atcttgcatt tttagtagtt gatattaagt tgatgacttg tttcccttca aggaaacatt 5160

Fig. 7B

aaattgtatg gactcagcta gctgttcaat gaaattgtga attagaaaca tttttaaaag 5220
tttttgaaag agataagtgc atcatgaatt acatgtacat gagaggagat agtgatatca 5280
gcataatgat tttgaggtca gtacctgagc tgtctaaaaa tatattatac aaactaaaat 5340
gtagatgaat taacctctca aagcacagaa tgtgcaagaa cttttgcatt ttaatcgttg 5400
taaactaaca gcttaaacta ttgactctat acctctaaag aattgctgct actttgtgca 5460
agaacttiga aggtcaaatt aggcaaattc cagatagtaa aacaatccct aagccttaag 5520
tctttttttt ttcctaaaaa ttcccataga ataaaattct ctctagttta cttgtgtgtg 5580
catacatctc atccacaggg gaagataaag atggtcacac aaacagtttc cataaagatg 5640
tacatattca ttatacttct gacctttggg ctttcttttc tactaagcta aaaattcctt 5700
tttatcaaag tgtacactac tgatgctgtt tgttgtactg agagcacgta ccaataaaaa 5760
tgttaacaaa atat 5774

Fig. 7C

1 80
slwliaaalvevrtsadgqagneemvqidlpikryreyelvtpvstnlegrylshtlsashkkrsardvssnpeqlffni
tafgkdfhlrlkpntqlvapgavvewhetslvpgnitdpinnhqpgsatyrirkteplqtncayvgdivdipgtsvaisn
cdglagmiksdneeyfieplergkqmeeekgrihvvykrsaveqapidmskdfhyresdlegldd1gtvygnihqqlnet
mrrrrhagendynievllgvddsvvrfhgkehvqnylltlmnivneiyhdeslgvhinvvlvrmimlgyaksisliergn
psrslenvcrwasqqqrsdlnhsehhdhaifltrqdfgpagmqqgyapvtgmchpvrsctlnhedgfssafvvahetghvl
gmehdgqgnrcgdetamgsvmaplvqaafhryhwsrcsgqelkryihsydcllddpfdhdwpklpelpginysmdeqcrf
dfgvgykmctafrtfdpckqlwcshpdnpyfcktkkgppldgtecaagkwcykghcmwknanqqkqdgnwgswtkfgscs
rtcgtgvrfrtrqcnnpmpinggqdcpgvnfeyqlcnteecqkhfedfraqqcqqrnshfeyqntkhhwlpyehpdpkkr
chlycqsketgdvaymkqlvhdgthcsykdpysicvrgecvkvgcdkeigsnkvedkcgvcggdnshcrtvkgtftrtpr
klgylkmfdippgarhvliqedeasphilaiknqatghyilngkgeeaksrtfidlgvewdynieddieslhtdgplhdp
vivliipqendtrssltykyiihedsvptinsnnviqeeldtfewalkswsqvskpcgggfqytkygcrrksdnkmvhrs
fceankkpkpirrmcniqecthplwvaeewehctktcgssgyqlrtvrclqplldgtnrsvhskycmgdrpesrrpcnrv
pcpaqwktgpwsecsvtcgegtevrqvlcragdhcdgekpesvracqlppcndepclgdksifcqmevlarycsipgynk
lccescskrsstlpppylleaaethddvisnpsdlprslvmptslvpyhsetpakkmslssissvggpnayaafrpnskp
dganlrqrsaqqagsktvrlvtvpsspptkrvhlssasqmaaasffaasdsigassqartskkdgkiidnrrptrsstle
r (1,201)

Fig. 8

GGAATTCGCGGCCGCGTCGACGTCAATACCAACTCCGAGCACACGGCCGTCATCAGCCTCTGCTCAGGAATGCTGGGCAC
ATTCCGGTCTCATGATGGGGATTATTTTATTGAACCACTACAGTCTATGGATGAACAAGAAGATGAAGAGGAACAAAACA
AACCCCACATCATTTATAGGCGCAGCGCCCCCCAGAGAGAGCCCTCAACAGGAAGGCATGCATGTGACACCTCAGAACAC
AAAAATAGGCACAGTAAAGACAAGAAGAAAACCAGAGCAAGAAAATGGGGAGAAAGGATTAACCTGGCTGGTGACGTAGC
AGCATTAAACAGCGGCTTAGCAACAGAGGCATTTTCTGCTTATGGTAATAAGACGGACAACACAAGAGAAAAGAGGACCC
ACAGAAGGACAAAACGTTTTTTATCCTATCCACGGTTTGTAGAAGTCTTGGTGGTGGCAGACAACAGAATGGTTTCATAC
CATGGAGAAAACCTTCAACACTATATTTTAACTTTAATGTCAATTGATGGGCCTTCCATATCTTTTAATGCTCAGACAAC
ATTAAAAAACCTTTGCCAGTGGCAGCATTCGAAGAACAGTCCAGGTGGAATCCATCATGATACTGCTGTTCTCTTAACAA
GACAGGATATCTGCAGAGCTCACGACAAATGTGATACCTTAGGCCTGGCTGAACTGGGAACCATTTGTGATCCCTATAGA
AGCTGTTCTATTAGTGAAGATAGTGGATTGAGTACAGCTTTTACGATCGCCCATGAGCTGGGCCATGTGTTTAACATGCC
TCATGATGACAACAACAAATGTAAAGAAGAAGGAGTTAAGAGTCCCCAGCATGTCATGGCTCCAACACTGAACTTCTACA
CCAACCCCTGGATGTGGTCAAAGTGTAGTCGAAAATATATCACTGAGTTTTTAGACACTGGTTATGGCGAGTGTTTGCTT
AACGAACCTGAATCCAGACCCTACCCTTTGCCTGTCCAACTGCCAGGCATCCTTTACAACGTGAATAAACAATGTGAATT
GATTTTTGGACCAGGTTCTCAGGTGTGCCCATATATGATGCAGTGCAGACGGCTCTGGTGCAATAACGTCAATGGAGTAC
ACAAAGGCTGCCGGACTCAGCACACACCCTGGGCCGATGGGACGGAGTGCGAGCCTGGAAAGCACTGCAAGTATGGATTT
TGTGTTCCCAAAGAAATGGATGTCCCCGTGACAGATGGATCCTGGGGAAGTTGGAGTCCCTTTGGAACCTGCTCCAGAAC
ATGTGGAGGGGGCATCAAAACAGCCATTCGAGAGTGCAACAGACCAGAACCAAAAAATGGTGGAAAATACTGTGTAGGAC
GTAGAATGAAATTTAAGTCCTGCAACACGGAGCCATGTCTCAAGCAGAAGCGAGACTTCCGAGATGAACAGTGTGCTCAC
TTTGACGGGAAGCATTTTAACATCAACGGTCTGCTTCCCAATGTGCGCTGGGTCCCTAAATACAGTGGAATTCTGATGAA
GGACCGGTGCAAGTTGTTCTGCAGAGTGGCAGGGAACACAGCCTACTATCAGCTTCGAGACAGAGTGATAGATGGAACTC
CTTGTGGCCAGGACACAAATGATATCTGTGTCCAGGGCCTTTGCCGGCAAGCTGGATGCGATCATGTTTTAAACTCAAAA
GCCCGGAGAGATAAATGTGGGGTTTGTGGTGGCGATAATTCTTCATGCAAAACAGTGGCAGGAACATTTAATACAGTACA
TTATGGTTACAATACTGTGGTCCGAATTCCAGCTGGTGCTACCAATATTGATGTGCGGCAGCACAGTTTCTCAGGGGAAA
CAGACGATGACAACTACTTAGCTTTATCAAGCAGTAAAGGTGAATTCTTGCTAAATGGAAACTTTGTTGTCACAATGGCC
AAAAGGGAAATTCGCATTGGGAATGCTGTGGTAGAGTACAGTGGGTCCGAGACTGCCGTAGAAAGAATTAACTCAACAGA
TCGCATTGAGCAAGAACTTTTGCTTCAGGTTTTGTCGGTGGGAAAGTTGTACAACCCCGATGTACGCTATTCTTTCAATA
TTCCAATTGAAGATAAACCTCAGCAGTTTTACTGGAACAGTCATGGGCCATGGCAAGCATGCAGTAAACCCTGCCAAGGG
GAACGGAAACGAAAACTTGTTTGCACCAGGGAATCTGATCAGCTTACTGTTTCTGATCAAAGATGCGATCGGCTGCCCCA
GCCTGGACACATTACTGAACCCTGTGGTACAGACTGTGACCTGAGGTGGCATGTTGCCAGCAGGAGTGAATGTAGTGCCC

Fig. 9A

AGTGTGGCTTGGGTTACCGCACATTGGACATCTACTGTGCCAAATATAGCAGGCTGGATGGGAAGACTGAGAAGGTTGAT
GATGGTTTTTGCAGCAGCCATCCCAAACCAAGCAACCGTGAAAAATGCTCAGGGGAATGTAACACGGGTGGCTGGCGCTA
TTCTGCCTGGACTGAATGTTCAAAAAGCTGTGACGGTGGGACCCAGAGGAGAAGGGCTATTTGTGTCAATACCCGAAATG
ATGTACTGGATGACAGCAAATGCACACATCAAGAGAAAGTTACCATTCAGAGGTGCAGTGAGTTCCCTTGTCCACAGTGG
AAATCTGGAGACTGGTCAGAGTGCTTGGTCACCTGTGGAAAAGGGCATAAGCACCGCCAGGTCTGGTGTCAGTTTGGTGA
AGATCGATTAAATGATAGAATGTGTGACCCAGAGGTCGACGCGGCCGCGAATTCCGCCGATACTGACGGGCTCCAGGAGT
CGTCGCCACCAATCCCCATATGGAAACCGTCGATATTCAGCCATGTGCCTTCAAGCCGAATTCCAG

Fig. 9B

GIRGRVDVNTNSEHTAVISLCSGMLGTFRSHDGDYFIEPLQSMDEQEDEEEQNKPHIIYRRSAPQREPSTGRHACDTSEH
KNRHSKDKKKTRARKWGERINLAGDVAALNSGLATEAFSAYGNKTDNTREKRTHRRTKRFLSYPRFVEVLVVADNRMVSY
HGENLQHYILTLMSIDGPSISFNAQTTLKNLCQWQHSKNSPGGIHHDTAVLLTRQDICRAHDKCDTLGLAELGTICDPYR
SCSISEDSGLSTAFTIAHELGHVFNMPHDDNNKCKEEGVKSPQHVMAPTLNFYTNPWMWSKCSRKYITEFLDTGYGECLL
NEPESRPYPLPVQLPGILYNVNKQCELIFGPGSQVCPYMMQCRRLWCNNVNGVHKGCRTQHTPWADGTECEPGKHCKYGF
CVPKEMDVPVTDGSWGSWSPFGTCSRTCGGGIKTAIRECNRPEPKNGGKYCVGRRMKFKSCNTEPCLKQKRDFRDEQCAH
FDGKHFNINGLLPNVRWVPKYSGILMKDRCKLFCRVAGNTAYYQLRDRVIDGTPCGQDTNDICVQGLCRQAGCDHVLNSK
ARRDKCGVCGGDNSSCKTVAGTFNTVHYGYNTVVRIPAGATNIDVRQHSFSGETDDDNYLALSSSKGEFLLNGNFVVTMA
KREIRIGNAVVEYSGSETAVERINSTDRIEQELLLQVLSVGKLYNPDVRYSFNIPIEDKPQQFYWNSHGPWQACSKPCQG
ERKRKLVCTRESDQLTVSDQRCDRLPQPGHITEPCGTDCDLRWHVASRSECSAQCGLGYRTLDIYCAKYSRLDGKTEKVD
DGFCSSHPKPSNREKCSGECNTGGWRYSAWTECSKSCDGGTQRRRAICVNTRNDVLDDSKCTHQEKVTIQRCSEFPCPQW
KSGDWSECLVTCGKGHKHRQVWCQFGEDRLNDRMCDPEVDAAANSADTDGLQESSPPIPIWKPSIFSHVPSSRIP

Fig. 10

cacatatgcacgagagagacagaggaggaaagagacagagacaaaggcacagcggaagaaggcagagacagggcaggcac
agaagcggcccagacagagtcctacagagggagaggccagagaagctgcagaagacacaggcagggagagacaaagatcc
aggaaaggagggctcaggaggagagtttggagaagccagacccctgggcacctctcccaagcccaaggactaagttttct
ccatttcctttaacggtcctcagcccttctgaaaactttgcctctgaccttggcaggagtccaagcccccaggctacaga
gaggagctttccaaagctagggtgtggaggacttggtgccctagacggcctcagtccctcccagctgcagtaccagtgcc
atgtcccagacaggctcgcatcccgggaggggcttggcagggcgctggctgtggggagcccaaccctgcctcctgctccc
cattgtgccgctctcctggctggtgtggctgcttctgctactgctggcctctctcctgccctcagcccggctggccagcc
ccctcccccgggaggaggagatcgtgtttccagagaagctcaacggcagcgtcctgcctggctcgggcacccctgccagg
ctgttgtgccgcttgcaggcctttggggagacgctgctactagagctggagcaggactccggtgtgcaggtcgaggggct
gacagtgcagtacctgggccaggcgcctgagctgctgggtggagcagagcctggcacctacctgactggcaccatcaatg
gagatccggagtcggtggcatctctgcactgggatgggggagccctgttaggcgtgttacaatatcgggggggctgaactc
cacctccagcccctggagggaggcaccctaactctgctgggggacctggggctcacatcctacgccggaagagtcctgc
cagcggtcaaggtcccatgtgcaacgtcaaggctcctcttggaagccccagccccagaccccgaagagccaagcgctttg
cttcactgagtagatttgtggagacactggtggtggcagatgacaagatggccgcattccacggtgcggggctaaagcgc
tacctgctaacagtgatggcagcagcagccaaggccttcaagcacccaagcatccgcaatcctgtcagcttggtggtgac
tcggctagtgatcctggggtcaggcgaggaggggccccaagtggggcccagtgctgcccagaccctgcgcagcttctgtg
cctggcagcggggcctcaacacccctgaggactcggaccctgaccactttgacacagccattctgtttacccgtcaggac
ctgtgtggagtctccacttgcgacacgctgggtatggctgatgtgggcaccgtctgtgacccggctcggagctgtgccat
tgtggaggatgatgggctccagtcagccttcactgctgctcatgaactgggtcatgtcttcaacatgctccatgacaact
ccaagccatgcatcagtttgaatgggcctttgagcacctctcgccatgtcatggcccctgtgatggctcatgtggatcct
gaggagccctggtccccctgcagtgcccgcttcatcactgacttcctggacaatggctatgggcactgtctcttagacaa
accagaggctccattgcatctgcctgtgactttccctggcaaggactatgatgctgaccgccagtgccagctgaccttcg
ggcccgactcacgccattgtccacagctgccgccgccctgtgctgccctctggtgctctggccacctcaatggccatgcc
atgtgccagaccaaacactcgccctgggccgatggcacaccctgcgggcccgcacaggcctgcatgggtggtcgctgcct
ccacatggaccagctccaggacttcaatattccacaggctggtggctggggtccttggggaccatggggtgactgctctc
ggacctgtgggggtggtgtccagttctcctcccgagactgcacgaggcctgtcccccggaatggtggcaagtactgtgag
ggccgccgtacccgcttccgctcctgcaacactgaggactgcccaactggctcagccctgaccttccgcgaggagcagtg
tgctgcctacaaccaccgcaccgacctcttcaagagcttcccagggcccatggactgggttcctcgctacacaggcgtgg
ccccccaggaccagtgcaaactcacctgccaggcccgggcactgggctactactatgtgctggagccacgggtggtagat

Fig. 11A

gggacccccctgttccccggacagctcctcggtctgtgtccagggccgatgcatccatgctggctgtgatcgcatcattgg
ctccaagaagaagtttgacaagtgcatggtgtgcggaggggacggttctggttgcagcaagcagtcaggctccttcagga
aattcaggtacggatacaacaatgtggtcactatccccgcgggggccacccacattcttgtccggcagcagggaaaccct
ggccaccggagcatctacttggccctgaagctgccagatggctcctatgccctcaatggtgaatacacgctgatgccctc
ccccacagatgtggtactgcctggggcagtcagcttgcgctacagcggggccactgcagcctcagagacactgtcaggcc
atgggccactggcccagcctttgacactgcaagtcctagtggctggcaacccccaggacacacgcctccgatacagcttc
ttcgtgccccggccgacccccttcaacgccacgccccactccccaggactggctgcaccgaagagcacagattctggagat
ccttcggcggcgcccctgggcgggcaggaaataacctcactatcccggctgccctttctgggcaccggggcctcggactt
agctgggagaaagagagagcttctgttgctgcctcatgctaagactcagtggggaggggctgtgggcgtgagacctgccc
ctcctctctgccctaatgcgcaggctggccctgccctggtttcctgccctgggaggcagtgatgggttagtggatggaag
gggctgacagacagccctccatctaaactgccccctctgccctgcgggtcacaggagggaggggaaggcagggagggcc
tgggccccagttgtatttatttagtatttattcacttttatttagcaccagggaaggggacaaggactagggtcctgggg
aacctgacccctgacccctcatagccctcaccctggggctaggaaatccagggtggtggtgataggtataagtggtgtgt
gtatgcgtgtgtgtgtgtgtgtgaaaatgtgtgtgtgcttatgtatgaggtacaacctgttctgctttcctcttcctgaa
ttttatttttgggaaaagaaaagtcaagggtagggtgggccttcagggagtgagggattatctttttttttttttcttt
ctttctttctttttttttttgagacagaatctcgctctgtcgcccaggctggagtgcaatggcacaatctcggctcact
gcatcctccgcctcccgggttcaagtgattctcatgcctcagcctcctgagtagctgggattacaggctcctgccaccac
gcccagctaatttttgttttgttttgtttggagacagagtctcgctattgtcaccagggctggaatgatttcagctcact
gcaaccttcgccacctgggttccagcaattctcctgcctcagcctcccgagtagctgagattataggcacctaccaccac
gcccggctaatttttgtatttttagtagagacggggtttcaccatgttggccaggctggtctcgaactcctgaccttagg
tgatccactcgccttcatctcccaaagtgctgggattacaggcgtgagccaccgtgcctggccacgcccaactaatttt
gtatttttagtagagacagggtttcaccatgttggccaggctgctcttgaactcctgacctcaggtaatcgacctgcctc
ggcctcccaaagtgctgggattacaggtgtgagccaccacgcccggtacatatttttaaattgaattctactatttatg
tgatccttttggagtcagacagatgtggttgcatcctaactccatgtctctgagcattagatttctcatttgccaataat
aatacctcccttagaagtttgttgtgaggattaaataatgtaaataaagaactagcataac

Fig. 11B

MSQTGSHPGRGLAGRWLWGAQPCLLLPIVPLSWLVWLLLLLLASLLPSARLASPLPREEEIVFPEKLNGSVLPGSGTPAR
LLCRLQAFGETLLLELEQDSGVQVEGLTVQYLGQAPELLGGAEPGTYLTGTINGDPESVASLHWDGGALLGVLQYRGAEL
HLQPLEGGTPNSAGGPGAHILRRKSPASGQGPMCNVKAPLGSPSPRPRRAKRFASLSRFVETLVVADDKMAAFHGAGLKR
YLLTVMAAAAKAFKHPSIRNPVSLVVTRLVILGSGEEGPQVGPSAAQTLRSFCAWQRGLNTPEDSDPDHFDTAILFTRQD
LCGVSTCDTLGMADVGTVCDPARSCAIVEDDGLQSAFTAAHELGHVFNMLHDNSKPCISLNGPLSTSRHVMAPVMAHVDP
EEPWSPCSARFITDFLDNGYGHCLLDKPEAPLHLPVTFPGKDYDADRQCQLTFGPDSRHCPQLPPPCAALWCSGHLNGHA
MCQTKHSPWADGTPCGPAQACMGGRCLHMDQLQDFNIPQAGGWGPWGPWGDCSRTCGGGVQFSSRDCTRPVPRNGGKYCE
GRRTRFRSCNTEDCPTGSALTFREEQCAAYNHRTDLFKSFPGPMDWVPRYTGVAPQDQCKLTCQARALGYYYVLEPRVVD
GTPCSPDSSSVCVQGRCIHAGCDRIIGSKKKFDKCMVCGGDGSGCSKQSGSFRKFRYGYNNVVTIPAGATHILVRQQGNP
GHRSIYLALKLPDGSYALNGEYTLMPSPTDVVLPGAVSLRYSGATAASETLSGHGPLAQPLTLQVLVAGNPQDTRLRYSF
FVPRPTPSTPRPTPQDWLHRRAQILEILRRRPWAGRK

Fig. 12

Rat ADAMTS 5 DNA

```
ACTCACTATA GGGCTCGAGC GGCCGCCCGG GCAGGTCAGA GGCTCACTGG CAGCTCTCTA     60
GACCTGCGAC GCTGCTTCTA TTCCGGGTAT GTGAACGCGG AGCCAGACTC CTTTGCTGCT    120
GTAAGCCTAT GCGGGGGTCT CCGCGGAGCC TTTGGCTACC AAGGTGCGGA GTATGTCATT    180
AGCCCTCTGC CCAACACCAG CGCGCCTGAG GCGCAGCGTC ATAGCCAGGG CGCACACCTT    240
CTCCAGCGCC GGGGTGCTCC CGTAGGGCCT TCCGGAGACC CTACCTCTCG CTGCGGGGTG    300
GCCTCGGGCT GGAACCCCGC CATCCTGAGG GCCTTGGACC CTTATAAACC ACGGCGGACG    360
GGCGTGGGCG AAAGCCACAA CCGGCGCAGG TCTGGGCGCG CCAAGCGCTT CGTGTCTATA    420
CCACGGTACG TGGAGACACT GGTGGTGGCG GACGAGTCAA TGGTCAAGTT TCACGGCGCG    480
GATTTGGAAC ATTATCTGCT GACGCTGCTG GCCACGGCGG CGCGACTCTA CCGCCACCCC    540
AGCATCCTCA ACCCTATCAA CATCGTTGTG GTCAAGGTGT TACTCTTAGG AGATCGTGAC    600
ACTGGGCCCA AGGTCACAGG CAACGCGGCC CTGACTCTGC GCAACTTCTG TGCCTGGCAG    660
AAAAAGTTGA ACAAAGTGAG CGACAAGCAC CCCGAGTACT GGGACACAGC CATCCTCTTC    720
ACCAGACAGG ACCTATGCGG GGCTACCACC TGTGACACCT TGGGCATGGC TGATGTGGGC    780
ACCATGTGTG ATCCCAAGAG AAGCTGCTCT GTCATCGAGG ACGATGGGCT TCCGTCGGCC    840
TTCACCACTG CCCATGAGCT GGGCCATGTG TTCAACATGC CCCATGACAA CGTGAAGGTG    900
TGTGAGGAGG TGTTTGGGAA GCTCAGAGCC AACCACATGA TGTCTCCGAC ACTCATCCAG    960
ATCGACCGTG CCAACCCCTG GTCAGCCTGC AGTGCTGCCA TTATCACCGA CTTCCTGGAC   1020
AGCGGGCACG GTGACTGCCT CCTGGACCAG CCCAGCAAGC CCATCACCCT GCCTGAGGAC   1080
CTGCCAGGCA CAAGCTACAG TTTGAGCCAA CAGTGCGAGC TGGCCTTTGG GGTGGGCTCT   1140
AAGCCCTGCC CATATATGCA GTACTGTACA AAGCTGTGGT GCACCGGCAA GGCCAAGGGG   1200
CAGATGGTGT GCCAGACTCG CCACTTCCCC TGGGCAGATG GCACCAGCTG TGGTGAGGGC   1260
AAGTTCTGCC TCAAGGGAGC CTGCGTGGAG AGACACAACC CAAACAAGTA CCGGGTGGAC   1320
GGCCCTTGGG CCAAGTGGGA GCCTTATGGT CCCTGCTCGC GCACCTGCGG TGGGGGCGCG   1380
CAGCTGGCCC GGAGGCAAGT GCAAGCAACC CTACCCCTGC CAACGGGCGG GAAGTACTGC   1440
GAGGGAGTGA GAGTGAAATA CCGATCTTGC AACTTGGAGC CCTGCCCCAG CTCAGCCTCT   1500
GGCAAGAGCT TCCGGGAA                                                 1518
```

Fig. 13

THYRARAAARAGQRLTGSSLDLRRCFYSGYVNAEPDSFAAVSLCGGLRGAFGYQGAEYVISPLPNTSAPEAQRHSQGAHL
LQRRGAPVGPSGDPTSRCGVASGWNPAILRALDPYKPRRTGVGESHNRRRSGRAKRFVSIPRYVETLVVADESMVKFHGA
DLEHYLLTLLATAARLYRHPSILNPINIVVVKVLLLGDRDTGPKVTGNAALTLRNFCAWQKKLNKVSDKHPEYWDTAILF
TRQDLCGATTCDTLGMADVGTMCDPKRSCSVIEDDGLPSAFTTAHELGHVFNMPHDNVKVCEEVFGKLRANHMMSPTLIQ
IDRANPWSACSAAIITDFLDSGHGDCLLDQPSKPITLPEDLPGTSYSLSQQCELAFGVGSKPCPYMQYCTKLWCTGKAKG
QMVCQTRHFPWADGTSCGEGKFCLKGACVERHNPNKYRVDGPWAKWEPYGPCSRTCGGGAQLARRQVQATLPLPTGGKYC
EGVRVKYRSCNLEPCPSSASGKSFR

Fig. 14

GATGCATCTAAGCCCTGGTCCAAATGCACTTCAGCCACCATCACAGAATTCCTGGATGATGGCCATGGTAACTGTTTGCT
GGACCTACCACGAAAGCAGATCCTGGGCCCCGAAGAACTCCCAGGACAGACCTACGATGCCACCCAGCAGTGCAACCTTA
CATTCGGGCCTGAGTACTCCGTGTGTCCCGGCATGGATGTCTGTGCTCCCCTGTGGTGTGCTGTGGTACGCCAGGGCCAG
ATGGTCTGTCTGACCAAGAAGCTTCCTGCGGTGGAAGGGACGCCTTGTGGAAAGGGGAGAATCTGCCTGCAGGGCAAATG
TGTGGACAAAACCAAGAAAAAATATTATTCAACGTCAAGCCATGGCAACTGGGGATCTTGGGGATCCTGGGGCCAGTGTT
CTCGCTCATGTGGAGGAGGAGTGCAGTTTGCCTATCGTCGCTGTAATAACCCTGCTCCCAGAAACAACGGACGCTACTGC
ACAGGGAAGAGGGCCATCTACCGCTCCTGCAGTCTCATGCCCTGCCCACCCAATGGTAAATCATTTCGTCATGAACAGTG
TGAGGCCAAAAATGGCTATCAGTCTGATGCAAAAGGAGTCAAAACTTTTGTGGAATGGGTTCCCAAATATGCAAGTGTCC
TGCCCAGCGATGTGTGCAAGCTGACCTGCAGAGCCAAAGGGACTGGCTACTATGTGGTATTTTCTCCAAAGGTGACCGAT
GGCACTGAATGTAGGCCGTACAGTAATTCCGTCTGCGTCCGGGGGAAGTGTGTGAGAACTGGCTGTGACGGCATCATTGG
CTCAAAGCTGCAGTATGACAAGTGCGGAGTATGTGGAGGAGACAACTCCAGCTGTACAAAGATTGTTGGAACCTTTAATA
AGAAAAGTAAGGGTTCANCTGACGTGGTGAGGATTCCTGAAGGGGCAACCCACATAAAAGTTCGACAGTTCAAAGCCAAA
GACCAGACTAGATTCACTGCCTATTTAGCCCTGAAAAAGAAAAACGGTGAGTACCTTATCAATGGAAAGTACATGATCTC
CACTTCAGAGACTATCATTGACATCAATGGAACAGTCATGAACTATAGCGGTTGGAGCCACAGGGATGACTTCCTGCATG
GCATGGGCTACTCTGCCACGAAGGAAATTCTAATAGTGCAGATTCTTGCAACAGACCCCACTAAACCATTAGATGTCCGT
TATAGCTTTTTTGTTCCCAAGAAGTCCACTCCAAAAGTAAACTCTGTCACTAGTCATGGCAGCAATAAAGTGGGATCACA
CACTTCGCAGCCGCAGTGGGTCACGGGCCCATGGCTCGCCTGCTCTAGGACCTGTGACACAGGTTGGCACACCAGAACGG
TGCAGTGCCAGGATGGAAACCGGAAGTTAGCAAAAGGATGTCCTCTCTCCCAAAGGCCTTCTGCGTTTAAGCAATGCTTG
TTGAAGAAATGTTAG

Fig. 15

DASKPWSKCTSATITEFLDDGHGNCLLDLPRKQILGPEELPGQTYDATQQCNLTFGPEYSVCPGMDVCAPLWCAVVRQGQ
MVCLTKKLPAVEGTPCGKGRICLQGKCVDKTKKKYYSTSSHGNWGSWGSWGQCSRSCGGGVQFAYRRCNNPAPRNNGRYC
TGKRAIYRSCSLMPCPPNGKSFRHEQCEAKNGYQSDAKGVKTFVEWVPKYASVLPSDVCKLTCRAKGTGYYVVFSPKVTD
GTECRPYSNSVCVRGKCVRTGCDGIIGSKLQYDKCGVCGGDNSSCTKIVGTFNKKSKGSXDVVRIPEGATHIKVRQFKAK
DQTRFTAYLALKKKNGEYLINGKYMISTSETIIDINGTVMNYSGWSHRDDFLHGMGYSATKEILIVQILATDPTKPLDVR
YSFFVPKKSTPKVNSVTSHGSNKVGSHTSQPQWVTGPWLACSRTCDTGWHTRTVQCQDGNRKLAKGCPLSQRPSAFKQCL
LKKC

Fig. 16

```
    M---------------------------------------   Majority
             10        20        30        40
1   M----------GDVQ-RAARS----------RGSLSAHML   mADAMTS-1
1   ----------------------------------------   hADAMTS-2
1   --GIR-----------------------------------   hADAMTS-3
1   LLGARQYRRNSGPPTPAPETSIANSKHPARLSRAAPPGAQ   rADAMTS-4
1   M----------SQTGSHPGRGLAGR----WLWGAQPCLLL   KIAA0688
1   SL--------------------------------------   KIAA0366
1   MDGRWQCS--------------------------------   KIAA0605

    -------------LLLLAL-TVLLSAD--AG-P---EEEL   Majority
             50        60        70        80
20  -------------LLLLASITMLLCARGAHGRPTEEDEEL   mADAMTS-1
1   ----------------------------------------   hADAMTS-2
4   ----------------------------------------   hADAMTS-3
41  RTMRLEWASLLLLLLLLCASCLALAADNPAAAPAQDKTRQ   rADAMTS-4
27  PIVPLSW---LVWLLLLLLASLLPSAR--LASPLPREEEI   KIAA0688
3   ------------WLIAAALVEVRTSADGQAGNEEMVQIDL   KIAA0366
9   -----CWAWFLLVLAVVAGDTVSTGSTDNSPTSNSLEGGT   KIAA0605

    V----P----------------LRG---P-G--GTTSRL-   Majority
             90       100       110       120
47  VL---PS--------------LERA---P-GHDSTTTRL-   mADAMTS-1
1   ----------------------------------------   hADAMTS-2
4   ----------------------------------------   hADAMTS-3
81  PR---AAAAAAQPDQRQWEETQERGHLQPLARQRRSSGLV   rADAMTS-4
62  VF---PE--------------KLNGSVLP-G-SGTPARLL   KIAA0688
31  PIKRYREYELVTPVSTNLEGRYLSHTLSASHKKRSARDVS   KIAA0366
44  DATAFW-----------WGEWTKWTAFSRSCGGGVTSQER   KIAA0605

    -NLD----------------G----L-LERDSGV-APG--   Majority
            130       140       150       160
65  -RLDAF--------------GQQLHLKLQPDSGFLAPGFT   mADAMTS-1
1   ----------------------------------------   hADAMTS-2
4   ----------GRVD--------------------------   hADAMTS-3
118 QNIDQLYSGGGKVGYLVYAGGRRFLLDLERDDTVGAAGGI   rADAMTS-4
83  CRLQAF--------------GETLLLELEQDSGVQVEGLT   KIAA0688
71  SNPEQLF-------FNITAFGKDFHLRLKPNTQLVAPGAV   KIAA0366
73  HCLQ------------------------QRRKSVPGPG--   KIAA0605
```

Fig. 17A